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Job Characteristics and Job Performance among Professional Nurses in the University Hospitals of People’s Republic of China

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ABSTRACT

This descriptive correlational study aimed to describe the level of job characteristics and job performance and to examine the relationship between job characteristics and job performance among professional nurses in two randomly- selected university hospitals in China. Data were collected from 328 professional nurses from a total population of 1,672, using stratified random sampling. Research instrument was composed of three parts: Demographic Data Form, Job Diagnostic Survey (JDS) and the Six-Dimension Scale of Nursing Performance (Six-D Scale). Reliabilities of the JDS and the Six-D Scale were .86 and .96, respectively. Descriptive statistics and Pearson’s product moment were used for data analysis. Findings showed that: 1) the level of overall job characteristics as perceived by professional nurses was at a moderate level. Among the seven dimensions of job characteristics, the level of job characteristics for dealing with others, task significance, feedback from agents and feedback from job itself were at a high level. The other dimensions, i.e., task identity, skill variety and autonomy were at a moderate level; 2) The quality of job performance in each dimension and overall job performance were at a moderate level and; 3) Among the subjects, a significant relationship was found between job characteristics and job performance that was at a moderate level (r=.36, p< .01). The results of this study can be used in planning work and training programs for professional nurses to ensure high level of job performance.

Key words: Job characteristics, Job performance, Professional nurses, University hospitals
INTRODUCTION

Healthcare organizations are changing amid economic development and the rapid reforms of healthcare systems, combined with scientific advances and consumers’ demand for better care (Lawler and Mohrman, 2000). Providing cost-effective and high quality healthcare is a key to achieving the goals of healthcare organizations (Hamilton et al., 2007) and is reliant on effective job performance by healthcare professionals.

As the Chinese healthcare delivery system reform strategies are implemented to increase overall cost-effectiveness (MOH, China, 2005), one reform method is to shorten patient’s hospital stay. Quality healthcare relies on the maintenance of a sufficient and qualified health care workforce, including nurses who form the second largest work group in healthcare. Job performance of nurses is defined as the level of effectiveness of a nurse in carrying out his or her roles and responsibilities related to direct nursing care and quality of healthcare services (Schwirian, 1978). Schwirian (1978) developed the six-dimension scale to measure aspects of nursing job performance which are: leadership, critical care, planning/evaluation, teaching/cooperation, interpersonal relationship/communication and professional development.

Nursing leadership is an important dimension of job performance. However, Chinese nurses generally have a low perception of the value of leadership and management skills which are not taught (Lu et al., 2007). The critical care dimension was perceived to be higher than the other dimensions of job performance among Chinese nurses (Yang et al., 2006). However, reports from the general public have rated nursing critical care as unsatisfactory, particularly among long-term and elderly patients (Ma, 2008). Chen and Huang (2006) studied the problems of quality of care in annual hospital inspection and reported that noted Chinese nurses did not perceive that planning and evaluation were important in nursing care. Teaching and collaboration generally have a low priority in Chinese hospitals, compared with critical care. Chinese nurses lack skills and the ability to effectively communicate with others (Jiang, 2008), however, interpersonal relationship and communication skills are vital to effective job performance. The employment of new medical technology and instruments requires continuous educational training for nurses to maintain and improve their level of job performance (Ma and Jiang, 2007). Professional development among nurses is restricted in contemporary China. Nurses have less opportunity to be trained and to develop new knowledge compared to physicians.

Job characteristics are all factors of the job and are directly associated with employee attitudes and behaviors at work (Hackman and Oldham, 1976). Hackman and Oldham stated that jobs with more challenges and variety inspired employees to improve their job skills and attitudes. This inferred a link between job characteristics and job performance. Edgar (1999) perceived that job characteristics affected nurses’ attitude, which, in turn, affected work outcomes, i.e., job performance. In China, the nursing work environment evolved with the change to patient-centered care (MOH, China, 2008) multiplying the role of nurses, thus affecting the design of nursing work.
Moreover, the university hospitals have implemented an annual assessment of job performance for all staff following the general national performance evaluation criterion (SAH, 2006, November). However, the hospital job performance appraisal form is too subjective to assess job performance of professional nurses. Furthermore, nurses stated that there was little feedback from nurse administrators on their performances (SAH, 2008, November).

Although many studies related to the job performance and job characteristics of nurses have been carried out worldwide (Hackman and Oldham, 1976; Edgar, 1999; Ang and Slaughter, 2001; Chua, 2006), no studies have been conducted in China. There is limited information on the effectiveness of nurses in carrying out their roles and responsibilities and how job characteristics influence job performance. The relationship between job characteristics and job performance among Chinese nurses remains undefined, and thus, is the rationale for this study.

**MATERIALS AND METHODS**

This descriptive correlational research aimed to examine the level of job characteristics and the level of job performance, and to determine the relationships between job characteristics and job performance among professional nurses in the university hospitals of Yunnan Province, P. R. China. The samples were 355 subjects from 1,672 professional nurses from two university hospitals in Yunnan Province, P. R. China.

Instrument used included three parts, the Demographic Data Form (DDF), the Job Diagnostic Survey (JDS) and the Chinese version of Six-Dimension Scale (Chinese Six-D Scale). The DDF was used to gather basic demographic information of each participant. It consisted of gender, age, marital status, educational level, present position, working experience as a registered nurse, department/section, professional title and employment type. The job characteristics were measured by the JDS which was originally developed by Hackman and Oldham (1975). The JDS consisted of 21 items in 7 dimensions: skill variety, task identity, autonomy, task significance, feedback from job itself, feedback from agents and dealing with others. Job performance was measured by the Chinese version of Six-Dimension Scale of nursing performance by Yang et al., (2006) which was based on Schwirian’s Six-Dimension Scale of nursing performance. This consisted of 52 items grouped into six dimensions which included: leadership, critical care, teaching/collaboration, planning/evaluation, interpersonal relationship/communication and professional development. The average score of nursing job performance in each dimension was categorized into three levels: low, moderate and high.

The Job Diagnostic Survey (JDS) was translated into Chinese by the researcher and back translated into English by two independent bilingual experts. The internal consistency and reliability of the instruments were pretested with 20 nurses within one of the university hospitals used in the study with the same criteria as the subjects at the study settings. In terms of job characteristics and job performance, the internal consistency of Cronbach’s alpha was calculated as .86 and .96, respectively.
After receiving the approval from the ethics committee of Faculty of Nursing, Chiang Mai University and the permissions from the administrative authorities at the university hospitals of Yunnan Province in China for data collection, the researcher distributed 355 questionnaires to the participants with consent forms. Three weeks after dissemination, the questionnaires were returned with a response rate of 100%, with 92.4% (328) completed and were used for data analysis.

The level of job characteristics and job performance were summarized, using descriptive statistics by the frequency, percentage, mean and standard deviation. The relationship between job characteristics and job performance was analyzed by using Pearson’s Product-Moment Correlation Coefficient.

RESULTS

The age of 328 professional nurses ranged from 21 to 54, with the majority of subjects being married (65.2%) and were female (97.6%). Most subjects held a bachelor’s degree of nursing qualification, only three (1.0%) held a master’s degree. The majority of the respondents were working as staff nurses and the remainder as managers. The largest group of participants held senior nurse (Hu Li Shi) professional title with an average length of work experience of 11.50 years (SD=8.25). Most participants worked in the surgical (43.0%) or medical (32.3%) departments, a small proportion in OB-GYN (4.3%) and pediatric (2.4%) departments and the remaining (18.0%) worked in other clinical departments. The largest group of subjects (54.0%) worked on rotating shifts and 50.3% worked in a permanent position.

The results indicated that the overall level of job characteristics in the university hospitals of Yunnan Province as perceived by professional nurses was at a moderate level with a mean score of 4.97 (SD=.83). Among the seven dimensions of job characteristics, the levels of job characteristics for dealing with others, task significance, feedback from agents and feedback from job itself were at a high level. The other dimensions including task identity, skill variety and autonomy were at a moderate level (Table 1).
Table 1. Mean, standard deviation and the level of job characteristics as perceived by professional nurses overall and in each dimension (n=328)

<table>
<thead>
<tr>
<th>Dimension of job characteristics</th>
<th>Mean (x̄)</th>
<th>SD</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skill variety</td>
<td>4.55</td>
<td>1.32</td>
<td>Moderate</td>
</tr>
<tr>
<td>Task identity</td>
<td>4.60</td>
<td>1.22</td>
<td>Moderate</td>
</tr>
<tr>
<td>Task significance</td>
<td>5.23</td>
<td>1.18</td>
<td>High</td>
</tr>
<tr>
<td>Autonomy</td>
<td>4.42</td>
<td>1.27</td>
<td>Moderate</td>
</tr>
<tr>
<td>Feedback from job itself</td>
<td>5.08</td>
<td>1.21</td>
<td>High</td>
</tr>
<tr>
<td>Feedback from agents</td>
<td>5.20</td>
<td>1.19</td>
<td>High</td>
</tr>
<tr>
<td>Dealing with others</td>
<td>5.70</td>
<td>1.13</td>
<td>High</td>
</tr>
<tr>
<td>Overall job characteristics</td>
<td>4.97</td>
<td>.83</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

As shown in Table 2, the average scores were calculated on each of the 4-point rating subscales. The results indicated that the level of job performance of professional nurses in each dimension and overall was at a moderate level.

Table 2. Mean standard deviation and level of quality of job performance in each dimension as perceived by professional nurses

<table>
<thead>
<tr>
<th>Dimension of job performance</th>
<th>Number of cases(n)</th>
<th>Mean</th>
<th>SD</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leadership</td>
<td>325</td>
<td>2.81</td>
<td>.53</td>
<td>Moderate</td>
</tr>
<tr>
<td>Critical care</td>
<td>326</td>
<td>2.87</td>
<td>.57</td>
<td>Moderate</td>
</tr>
<tr>
<td>Teaching/collaboration</td>
<td>322</td>
<td>2.56</td>
<td>.51</td>
<td>Moderate</td>
</tr>
<tr>
<td>Planning/evaluation</td>
<td>319</td>
<td>2.67</td>
<td>.53</td>
<td>Moderate</td>
</tr>
<tr>
<td>Interpersonal relationship/communication</td>
<td>327</td>
<td>2.84</td>
<td>.46</td>
<td>Moderate</td>
</tr>
<tr>
<td>Overall job performance</td>
<td>328</td>
<td>2.82</td>
<td>.42</td>
<td>Moderate</td>
</tr>
</tbody>
</table>
The results also indicated that job characteristics were significantly associated with overall and each dimension of job performance among professional nurses (Table 3).

**Table 3.** Pearson’s Correlation matrix of job characteristics and each dimension of job performance and overall job performance

<table>
<thead>
<tr>
<th>Job performance</th>
<th>Job characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Leadership</td>
<td>325</td>
</tr>
<tr>
<td>Critical care</td>
<td>326</td>
</tr>
<tr>
<td>Teaching/collaboration</td>
<td>322</td>
</tr>
<tr>
<td>Planning/evaluation</td>
<td>319</td>
</tr>
<tr>
<td>Interpersonal relationship/communication(IPR)</td>
<td>327</td>
</tr>
<tr>
<td>Professional development</td>
<td>328</td>
</tr>
<tr>
<td>Overall job performance</td>
<td>314</td>
</tr>
</tbody>
</table>

Note. r=Pearson’s coefficient between job characteristics and each dimension of job performance.

**.p<0.01

**DISCUSSION AND CONCLUSION**

The findings of this study indicated that the current job characteristics provided the respondents with a moderate level of motivation in carrying out their duties. When compared to the results of previous studies, there was consistency with in the study by Peng and Li (2008). However, they were not congruent with prior studies in Macao (Guo, 2007) and from western countries where the job characteristics were perceived at a “high level” (Edgar, 1999; Hall and Pallas, 2000). These findings may be due to different settings, ethnic composition and socio-economic environments and the different evolutions of hospital management structures and care models. One possible explanation for the results of the study is that as China embarks on a new round of health-care reforms, effective policies are needed to better manage nursing. Most nursing jobs are designed to improve effectiveness, however, many policies from the central authority are not implemented satisfactorily in remote provinces (Peng and Li, 2008). In addition, Yunnan province is located in southwest of China and is perceived as one of the developed province when compared to nationwide China (YPBH, 2008 May), hence, proper attention has not been paid to developing effective job characteristics. The job description for professional nurses in the university hospitals may be perceived by nurses as being poorly designed, and the jobs were regarded as being simple and repetitive (YPBH, 2008 May).

This study found that job performance was perceived at a moderate level by professional nurses (Table 2). This may be due to work experience, education level and professional title. In the study, most of the professional nurses (32.9%)
had work experience from one to five years. New nurses may lack the experience to provide effective nursing care for patients, since their job performance was based on work experience and still continuing to develop nursing skills (Li et al., 2001).

The study found that job characteristics were positively related to overall and to each of the six dimensions of job performance: leadership, critical care, teaching/collaboration, planning/evaluation, interpersonal relationship/communication, and professional development (Table 3). The relationship between job characteristics and job performance among professional nurses in this study was congruent with the findings from previous studies conducted by Hackman and Oldham (1976) and Panzano et al., (2006). Job performance is expected to be increased when the job provides employees with an opportunity to make decisions about how and when to do tasks (Yang et al., 2006). Thus, if professional nurses are motivated to provide an efficient and quality nursing care service, the productivity and performance of the hospital will be improved.

Job characteristics were positively related to leadership in the six dimensions of performance that demonstrated a positive link between leadership and professional nurses’ perception of their job. This may be because professional nurses who perceived their job as more challenging, meaningful and significant were engaged in significant leadership responsibilities (Edgar, 1999). The study also found that job characteristics were positively related to the critical care and planning/evaluation dimensions. The results could be explained that professional nurses who provide critical care must use nursing processes, with planning/evaluation is integral responsibility. A simple and routine job reduces motivation to perform well whereas a challenging job increases motivation (Ramlall, 2004). The relationship between teaching/cooperation, interpersonal relationship/communication and the characteristics of job was significantly correlated. This suggested that when professional nurses perceived a high level of feedback from the job and when the hospital’s administration and nurse managers have the well-established performance criteria, this would enable professional nurses to evaluate themselves and create good work outcomes. Hence, the more feedback from the job and agents, the more the effectiveness of cooperation and communication among professional nurses increased.

The findings also showed that there were a positive relationship between job characteristics and professional development dimension of job performance. It might be explained as, when professional nurses understand the characteristics of the nursing job, they may develop a strong positive attitude towards their work. This may contribute to the development of the hospital services and nursing profession (Lu et al., 2007). The quantity of enrollments in professional development programs by nurses will increase further, boosting nursing skills. Autonomy, significance and meaningfulness in nursing work play an important role in the nurses’ perception of their jobs (Edgar, 1999; Peng and Li, 2008). This may result in more meaningful rewards and recognition of the nursing profession from the hospital administration and China society, thus a more significant perception of the value of professional nurses.
The study findings have demonstrated a general consistency with previous studies in terms of the level of job characteristics (Peng and Li, 2008). The level of job performance was also found to be generally consistent with previous studies, with critical care at a moderate level (Yang et al., 2006). The relationship between job characteristics and job performance as reported by Edgar (1999) was found to exist at a moderate level in four of the six job performance dimensions. Maintaining a high level of job performance is vital to maintaining the effectiveness of the healthcare provided by professional nurses.

IMPLICATIONS

The findings of this study support the job characteristics model and found a linkage between job characteristics and job performance among professional nurses. The results have implications for nursing administration, nursing practice and for hospital policy makers in planning work and training programs for professional nurses.

RECOMMENDATIONS FOR FURTHER STUDY

The study of job performance of professional nurses by using other methods, such as patients and peers evaluation or observed study should be conducted. Moreover, intervention study of job performance improvement should be carried out in future research.

ACKNOWLEDGEMENTS

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Yunnan Provincial Bureau of Health 2008, May. Committee meeting minutes, Kunming: Yunnan Provincial Bureau of Health (YPBH)
Comparison of Alternative Methods with Intensive Method for Collecting insects and spider Data in Rice Field

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ABSTRACT

Diversity of arthropods in rice field is an important index for determining biodiversity, species richness and their balance. In order to collect data for calculating the biodiversity index, the conventional intensive method is not easy for general people because of its complexity. Hence, methods with fewer complexes that farmers can use for rapid health impact assessment need to be developed. This study aimed to compare the alternative methods for collecting arthropods data with the intensive method. A field experimental study was conducted in the wet and dry season during September 2007-April 2009. A sweep net sampling method was used to collect insects from three rice fields: untreated, treated with pesticide at recommended rate and double rate, in the following four methods: 1) Intensive method by the International Rice Research Institute, 2) Thai Farmer School method, 3) Randomly 3-points, and 4) Randomly 1-point in the centre of plot. Total of 19,200 samples were collected within 19,200 m² from the 12 sites of two northeastern provinces of Thailand. The species richness index (Esn) and the exponential Shannon index (exp H’) were computed by EcoSim. The Esn and exp H’ differences that were considered ecologically meaningful. Sample sizes were equalized through rarefaction before comparison. Mean difference (MD) between groups with their 95% confident intervals were estimated using linear regression model. The results showed that Esn and exp H’ from the Thai Farmer School method was not significantly different from the Intensive method. This study demonstrated that the efficiency of Thai Farmer School method is comparable to the Intensive method, being easier, cheaper and more practical in farmer’s opinion.

Key words: Rice field, Shannon-Wiener index, Rarefaction, Biodiversity
INTRODUCTION

Thailand has a strong tradition of rice production and is the world’s top ranked rice exporters (10.22 million tons) in 2008. Moreover, Thailand has plans to further increase its land available for rice production, with a goal of adding 57.5 million rai to its already 58 million rai of rice-growing areas (AFSIS, 2009). The Thai Ministry of Agriculture expects rice production to yield around 529 kg/rai in wet season and 764 kg/rai in dry season for 2011 (AFSIS, 2009). Actually, in growing rice, pesticides may or may not be applied in rice field but pesticides are usually used for controlling pests such as brown planthopper (Nilaparvata lugens) (Escalada et al., 2009). Hence, the adverse effects arise from both direct and indirect human contacts (Huang et al., 2000). Especially indirect way, it impacts on diversity of plants, animals and microbe species (Ruayaree, 2002; Praneetvatakull and Waibel, 2006).

In order to collect data for calculating the biodiversity index and species richness, there are several intensive methods available including FARMCOP Suction machine (Cariño et al., 1979), Blower-vac machine (Arida and Heong, 1992), Yellow sticky trap, Water pan, Pitfall trap, Light trap, Yellow pan trap and Insect sweep net sampling method (International Rice Research Institute, 1981). Although highly efficient, these methods are difficult for general people due to their complexity, and some methods are expensive, labor- and time-consuming. Hence, other alternative methods which are more convenience to farmers and can be accepted by researchers in academics in sampling and collecting insects are needed to be considered.

Farmer school method, which was developed by Thai farmers, is a sweep net sampling method regularly used by farmers in Thailand. This method is relatively simple but its effectiveness has not yet been systematically evaluated. In this study, alternative sweep net sampling methods include Thai Farmer School Method that is more practical and cheaper than the intensive methods. In addition, the Thai farmer school method is more practical for farmer. Next, randomly 3-point method is currently used by academics in case of studying big area because of being time-saving and more comfortable to collect insects. Finally, randomly 1-point method in the center of the plot is the way that farmers tried to propose to collect insects in rice field because of being the most comfortable and the easiest method. Hence, the objective of this study was to develop alternative methods for collecting arthropods data and compare with the intensive method.

MATERIALS AND METHODS

Experimental sites

Six separate rice growing areas at Khon Kaen and Kalasin Province, Northeast of Thailand, were selected as experimental sites in the wet and dry seasons during September 2007-April 2009. Detailed descriptions of the experimental sites are presented in Table 1.
Table 1. Summary of experimental sites, Khon Kaen and Kalasin Provinces, Thailand

<table>
<thead>
<tr>
<th>Sites</th>
<th>Location, elevation</th>
<th>Rain patterns</th>
<th>Cropping pattern</th>
<th>Sampling dates, plot size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khon Kaen</td>
<td>17° 30′ N, 102° 25′ E</td>
<td>Annual rainfall May-September</td>
<td>- Rice mixed with vegetables</td>
<td>*May 16-August 7, 2008 (wet season)</td>
</tr>
<tr>
<td></td>
<td>900 m above sea level</td>
<td>- Control quantity of water by flowing open canal</td>
<td></td>
<td>*January 5-April 19, 2009 (dry season)</td>
</tr>
<tr>
<td></td>
<td>1,200 mm rain</td>
<td></td>
<td></td>
<td>3,200 m²</td>
</tr>
</tbody>
</table>

| Kalasin  | 16° 26′ N, 103° 30′ E | Annual rainfall May-September | - Rice mixed with vegetables                          | *May 30-August 25, 2008 (wet season) |
|          | 152 m above sea level | - Control quantity of water by flowing open canal   |                                                       | *January 14-April 30, 2009 (dry season) |
|          | 1,200 mm rain        |                        |                                                       | 3,200 m²                |

Sampling

Four methods of sweep net sampling, 1) Intensive method - the standard manual for testing insecticides on rice fields developed by the International Rice Research Institute, 2) Thai Farmer School method, 3) randomly 3-points, and 4) randomly 1-point in the centre of plot, were used to collect insects from the six rice fields under three pesticide treatments: untreated, treated pesticide at recommended rate and treated at double rate. Total of 19,200 samples were collected within 19,200 m² from the 12 sites, taken at weekly intervals. Sweep net sampling was replicated 10 times on each occasion at each site. Collected arthropods especially insects and spider were kept in vials of 70% ethanol. Samples in individual vial were sorted and counted together with farmers and then checked in the laboratory. The arthropods obtained from the samples were identified to species whenever possible. They were later grouped into guilds as used by Moran and Southwood (1982) and Heong et al., (1991).

Steps of collecting arthropods of 4 methods were as follows (see Fig. 1):

1) Intensive method (IM)
   1.1 Hold the sweep net near the end of the handle;
   1.2 Begin sweeping at the centre of the plot;
   1.3 Swing the pole with both arms forming a semicircle. Keep the circular frame of the open end of the net perpendicular to the ground and pointing to the direction of the swing;
   1.4 Walk normally and swing the net steadily, touching the leafy portion of the plant. Do not swing the net up and down. Close net opening as soon as sweeping action is completed;
   1.5 Sweep 10 times per plot;
   1.6 Put the collected insects in plastic bags and label with tags;
   1.7 Keep in vials of 70% ethanol;
   1.8 Identify and count the insects and spiders with farmers and in the laboratory.

2) Thai Farmer School method (THFM)
   2.1 Begin sweeping at the margin of the plot to another;
2.2 Swing the pole with both arms;
2.3 Walk normally and swing the net steadily, touching the leafy portion of the plant;
2.4 Put the collected insects and spiders in plastic bags and labels with tags;
2.5 Put the insect and spiders bags in hot water;
2.6 Identify and count the insects and spiders suddenly with farmers;
2.7 Keep in vials of 70% ethanol;
2.8 Identify and count the insects and spiders in laboratory.

3) Randomly 3-point method (TPM)
3.1 Hold the sweep net near the end of the handle;
3.2 Begin sweeping at the centre of the plot;
3.3 Swing the pole with both arms forming a semicircle. Keep the circular frame of the open end of the net perpendicular to the ground and pointing to the direction of the swing;
3.4 Walk normally and swing the net steadily touching the leafy portion of the plant. Do not swing the net up and down. Close net mouth as soon as sweeping action is completed;
3.5 Sweep 10 times per plot (from 4 corners and center of plot);
3.6 Put the collected insects and spiders in plastic bags and labels with tags;
3.7 Keep in vials of 70% ethanol;
3.8 Identify and count the insects and spiders with farmers and in the laboratory.

4) Randomly 1-point method- proposed from meeting with farmers (OPM)
4.1 Begin sweeping at the margin of the plot to another by three farmers per plot;
4.2 Swing the pole with both arms;
4.3 Walk normally and swing the net steadily, touching the leafy portion of the plant;
4.4 Put the collected insects and spiders in plastic bags and label with tags;
4.5 Put the insect and spiders bags in hot water;
4.6 Identify and count the insects and spiders suddenly with farmers;
4.7 Keep in vials of 70% ethanol;
4.8 Identify and count the insects and spiders in laboratory.
Insecticide application

Thiamethoxam 25% WG (Ac'tara®) was used to control insect pests in the sprayed rice field in the ratio of 10 grams per 20 litres of water (Maienfisch, 2006). Application timing was related to brown planthopper migration and at two stages of rice development. In wet season, the pesticide was first applied at vegetative stage in July 5, 2008 and then at reproductive stage in July 25 and July 31, 2008. In dry season the applications were done at vegetative stage in February 12, 2009 and at reproductive stage in March 10 and March 25, 2008.

Site Selection

Six sites were divided into three groups for Khon Kaen and Kalasin provinces as 1) untreated pesticide¹ or control group, 2) treated pesticide at recommended rate, and 3) treated at double rate. In group 2 and 3 various pesticides have been used thiamethoxam, cipermethrin, indoxacarb, monocrotophos on rice field more than 10 years. The reasons that we chose these sites because 1) there are both organic and conventional sites together. 2) there are good irrigation systems i.e. open canal that farmers can use water all the year. 3) these sites are continuously used for planting two times per year.

¹This site has stopped using pesticide for seven years and qualified as an organic farm under standard EU2092/91 No. CU 019946 for European countries and OMIC No. 1262 for Japan (2002- present) under which agricultural product are imported from European countries.
Quality control

1) Sweepers were well-trained with experience in the sweep method and insect identification.

2) The following factors were equally assigned to all experiment groups.
   • Soil type: Silt-loam
   • Fertilizer 15:15:15 (N:P:K)
   • Fertilizer rate: 80 kg. per 1,600 m²
   • Type of rice cultivation: direct seeding
   • Rice variety: KDML105
   • Size of experimental area: 1,600 m²
   • Cultural practice: land preparation, seed germination

Data analysis

The indices that were used in this study were as follows:
1. Rarefaction
   Rarefaction techniques were used to avoid sample size sensitivity by computing species richness. The less sample size sensitive indices with more discriminating abilities were used for comparison. The formula is as following:

\[
E_{sn} = \sum \left[ 1 - \left( \frac{N - Ni}{n} \right) \right]
\]

Where \( E_{sn} \) = the expected number of species in the rarefied sample
\( n \) = standard sample size
\( N \) = the total number of individuals recorded in the sample to be rarefied
\( Ni \) = the number of individuals in the ith species in the sample to be rarefied

Remark: the term \( \left( \frac{N - Ni}{n} \right) \) and \( \left( \frac{N}{n} \right) \) are ‘combinations’ which are as follows:

\[
\left( \frac{N}{n} \right) = \frac{N!}{n! \cdot (N-n)!}
\]

\( N! \) is a factorial. For example \( 4! = 4*3*2*1 = 24 \)

2. Shannon diversity index (H')
   The formula for calculating the H' is
   \[
   H' = -\sum pi \cdot \ln pi
   \]
   Where \( pi \), the proportional abundance of the ith species = \( (ni/N) \)
In calculating, \( \exp H' \), the exponential Shannon index, was transformed by taking exponential to Shannon-Wiener index before doing the comparison in order to provide more discriminating abilities (Magurran, 1988).

The functional biodiversity indices were analyzed using indices computed by EcoSim (Gotelli and Entsminger, 2005) -null model software for ecology.

Finally, mean difference (MD) between groups with 95% confident intervals was estimated by linear regression model.

**RESULTS**

The total of 4,830 arthropods were found in the experimental rice fields with intensive method which was a standard and could be sorted into 4 guilds as herbivores (41.57%), predators (23.98%), parasitoids (19.03%) and detritivores (15.42%) (Table 2). Although the number of herbivores or pests was very high in all method of collecting insects, the total number of beneficial insects and spiders including predators, parasitoids and detritivores was still higher than pests. It meant that the beneficial insects could control pests in these rice fields in both Khon Kaen and Kalasin provinces. During sorting, thrips, beetles and hoppers were found as the most herbivorous insects. The most of predaceous arthropods were spiders, hemipterans and beetles. The most of detritivorous arthropods were dipterans. According to this result, this rice field ecosystem was likely to be good for the food chain because it contained the beneficial insects especially predators and parasitoids, which are secondary consumers that feed on primary consumers. For example, lady beetle (Micraspis discolor) consumes brown planthopper (Nilaparvata lugens), and long-jawed spider (Tetragnatha spp.) eats both green leafhopper (Nephotettix virescens) and white leafhopper (Cofana spectra). These relationships created a balance among the remaining species.

All arthropods were collected by four methods as an intensive method, Thai farmer school method, randomly 3-point method, and randomly 1-point method. The results are shown in Table 2. The \( \exp H' \) and the \( E_{sn} \) of the arthropods guilds in Thai farmer school method were not significantly different from those indices in the intensive method and showed mean difference (MD) of the \( \exp H' \) and \( E_{sn} \) (rarefaction) of the overall arthropods guilds which was classified by functional group of arthropods.

The maximum of MD of \( \exp H' \) in intensive method and Thai farmer school method was shown in detritivores between dry season and treated pesticide with double rate in Khon Kaen which was 0.32 (0.21 to 0.43); means (M) ± standard deviation (SD): 3.58 ± 0.29 and 3.26 ± 0.23, respectively. Indices of both of the randomly 3-point and 1-point method were significantly different from the intensive method. The maximum of MD was shown in herbivores between wet season and untreated pesticide in Khon Kaen which was 1.05 (0.99 to 1.11); M ± SD: 9.34 ± 0.32 and 8.29 ± 0.23, respectively, for randomly 3-point method, and the maximum of MD was 3.87 (3.72 to 4.02); M ± SD: 9.34 ± 0.32 and 5.47 ± 0.97, respectively; \( p<0.001 \) for randomly 1-point method (Table 3).
Regarding the $E_{sn}$ (rarefaction), the maximum of MD in intensive method and Thai farmer school method was shown in parasitoids between wet season and untreated pesticide in Khon Kaen which was 0.3(-0.01 to 0.61); means (M) ± standard deviation (SD): 26.0 ± 1.03 and 25.7 ± 0.83, respectively; $p=0.059$. The maximum of MD of randomly 3-points and 1-point methods, was shown in pests between dry season and treated pesticide with recommended rate in Kalasin which was 4.1(3.11 to 5.09); M ± SD: 19.1 ± 0.74 and 15.0 ± 4.11, respectively; $p<0.001$ for randomly 3-points method, and the maximum of MD was 8.8(7.71 to 9.89); M ± SD: 19.1.6 ± 0.74 and 10.3 ± 4.57, respectively; $p<0.001$ for randomly 1-point method (Table 3).

**DISCUSSION AND CONCLUSION**

The results showed that the distribution pattern of insects per hill of rice plant has a general tendency to be aggregated or contagious. Kuno (1963), Kuno and Dyck (1985) and Kusmayadi et al., (1990) studied the population of brown planthoppers (BPHS) in the paddy fields of Japan, Philippines, and West Java, respectively, and found to be of non-random distribution. This is because the adult BPHS lay eggs as egg masses and several egg masses may be successively laid on the same hill. It is understandable that the distribution of offsprings would become patchy, even though the initial distribution of their parents is random (Kuno, 1968, 1977).

There was a tendency that nymphs distribute themselves more contagiously than adults (Kuno, 1968; Kusmayadi et al., 1990) because of no permanent wings to fly. Among adults, the degree of aggregation was higher in macropters than in brachypters. This difference may be a consequence of the density-dependent manner of wingmorph determination. The proportion of brachypterous form among emerging adults would be higher in hills which have been occupied by a small number of nymphs whereas the macropterus form would become dominant among hills with high nymphal density (Kisimoto, 1965; Kusmayadi et al., 1990; Barclay, 1992). These reasons were supported by the results from randomly 1-point and 3-points methods which showed relatively wide range of 95% CI of $E_{sn}$. The pattern of $E_{sn}$ was unpredictable. It was low when farmers swept net in contagious hills of rice plant with facing adult stage so they could fly to the other hills. But sometimes $E_{sn}$ was high when sweepers faced the aggregated hills with nymph and brachypterous stage. Hence, both randomly 3-point and 1-point methods were significantly different from the intensive method which $E_{sn}$ was quite contagious. In the same ways, $E_{sn}$ was contagious in Thai farmer school method which looks like intensive method because farmers could randomly sweep net in all rice fields.

In aspect of ecological meaning of the $E_{sn}$ difference, three ecologists, 1) Assist.Prof. Dr. Adcharaporn Pagdee 2) Assist. Prof. Sam-ang Homchurn and 3) Dr. Kong Luen Heong, said that this difference was small and acceptable in Thai farmer school method but it was much different in randomly 3-point and 1-point method because 1) It’s possible that farmers swept insects at different growing
Table 2. Comparison of methods of collecting arthropods by using exp Shannon-Wiener index and species richness in rice field in 2 provinces

<table>
<thead>
<tr>
<th>Methods</th>
<th>Exp H(^1)</th>
<th>E(_{\text{sn}}) (rarefaction)(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (adjusted)</td>
<td>MD(^2)</td>
</tr>
<tr>
<td>1) Herbivores</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Intensive method (n = 2008)</td>
<td>8.18 ± 2.70</td>
<td>0</td>
</tr>
<tr>
<td>- Thai farmer school method (n = 2025)</td>
<td>8.37 ± 2.79</td>
<td>-0.19</td>
</tr>
<tr>
<td>- Three point method (n = 1818)</td>
<td>7.80 ± 2.73</td>
<td>0.38</td>
</tr>
<tr>
<td>- One point method (n = 1630)</td>
<td>6.95 ± 2.62</td>
<td>1.23</td>
</tr>
<tr>
<td>2) Predators</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Intensive method (n = 1158)</td>
<td>8.37 ± 3.69</td>
<td>0</td>
</tr>
<tr>
<td>- Thai farmer school method (n = 1108)</td>
<td>8.33 ± 3.68</td>
<td>0.04</td>
</tr>
<tr>
<td>- Three point method (n = 965)</td>
<td>8.03 ± 3.63</td>
<td>0.34</td>
</tr>
<tr>
<td>- One point method (n = 843)</td>
<td>7.39 ± 3.45</td>
<td>0.98</td>
</tr>
<tr>
<td>3) Parasitoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Intensive method (n = 919)</td>
<td>7.95 ± 3.36</td>
<td>0</td>
</tr>
<tr>
<td>- Thai farmer school method (n = 846)</td>
<td>8.28 ± 3.59</td>
<td>-0.33</td>
</tr>
<tr>
<td>- Three point method (n = 802)</td>
<td>8.08 ± 3.54</td>
<td>-0.13</td>
</tr>
<tr>
<td>- One point method (n = 637)</td>
<td>7.39 ± 3.39</td>
<td>0.56</td>
</tr>
<tr>
<td>4) Detritivores</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Intensive method (n = 745)</td>
<td>4.72 ± 1.86</td>
<td>0</td>
</tr>
<tr>
<td>- Thai farmer school method (n = 721)</td>
<td>4.69 ± 1.85</td>
<td>0.03</td>
</tr>
<tr>
<td>- Three point method (n = 650)</td>
<td>4.35 ± 1.70</td>
<td>0.37</td>
</tr>
<tr>
<td>- One point method (n = 575)</td>
<td>3.84 ± 1.79</td>
<td>0.88</td>
</tr>
</tbody>
</table>

\(^1\)Analyzed by EcoSim (Gotelli and Entsminger, 2005)
\(^2\)MD=Mean Difference after rarefaction

Remark: n in Table 2 showed number of all insects and spiders.
Table 3. Highlight of maximum of mean difference in alternative methods comparing with intensive method under every conditions by using exp $H'$ and $E_{sn}$ (rarefaction) in rice field

<table>
<thead>
<tr>
<th>Methods</th>
<th>Exp $H'$</th>
<th>$E_{sn}$ (rarefaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD (adjusted)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1) Herbivores</td>
<td>$^{In<del>wet</del>season}$</td>
<td>Khon Kaen province Untreated site</td>
</tr>
<tr>
<td>- Intensive method</td>
<td>193</td>
<td>9.34 ± 0.32</td>
</tr>
<tr>
<td>- Thai farmer school method</td>
<td>204</td>
<td>9.46 ± 0.41</td>
</tr>
<tr>
<td>- Three point method</td>
<td>152</td>
<td>8.29 ± 0.23</td>
</tr>
<tr>
<td>- One point method</td>
<td>115</td>
<td>5.47 ± 0.97</td>
</tr>
</tbody>
</table>

| 2) Predators       |          |                        |         |              |                      |         |              |
|                    |          |                        |         |              |                      |         |              |
| - Intensive method | 135      | 6.37 ± 0.19            | 0       |              | 53.7 ± 0.48          | 0       |              |
| - Thai farmer school method | 127  | 6.12 ± 0.53            | 0.25    | 0.15 to 0.34  | 53.8 ± 0.42          | -0.1    | -0.27 to 0.69 |
| - Three point method | 129   | 6.13 ± 0.28            | 0.24    | 0.18 to 0.29  | 52.4 ± 1.5           | -0.1    | -0.25 to 0.06 |
| - One point method | 108      | 4.19 ±1.81             | 2.18    | 1.87 to 2.48  | 45.8 ± 4.29          | 3.2     | 2.47 to 3.93 |

| 3) Parasitoids     |          |                        |         |              |                      |         |              |
|                    |          |                        |         |              |                      |         |              |
| - Intensive method | 132      | 6.37 ± 0.19            | 0       |              | $^{26.0 ± 1.03}$     | 0       |              |
| - Thai farmer school method | 126  | 6.12 ± 0.53            | 0.25    | 0.15 to 0.34  | $^{25.7 ± 0.83}$     | $^{0.3^*}$ | -0.01 to 0.61 |
| - Three point method | 125   | 6.13 ± 0.28            | 0.24    | 0.18 to 0.29  | 25.8 ± 0.42          | 0.2     | -0.06 to 0.46 |
| - One point method | 70       | 4.19 ±1.81             | 2.18    | 1.87 to 2.49  | 22.1 ± 2.69          | 3.9     | 3.22 to 4.58 |

$^1$ Analyzed by EcoSim (Gotelli and Entsminger, 2005)

$^2$ MD = Mean Difference after rarefaction

* The maximum of MD in Farmer school method comparing with intensive method by using both exp $H'$ and $E_{sn}$ (rarefaction)

** The maximum of MD in randomly 3-points method and randomly 1-point method comparing with intensive method by using both exp $H'$ and $E_{sn}$ (rarefaction)

n = number of arthropods in rice field

Remark: n in Table 3 showed number of insects and spiders in specific condition which showed highlight of maximum only.
### 4) Detritivores

<table>
<thead>
<tr>
<th>Method</th>
<th>Intensive method</th>
<th>Thai farmer school method</th>
<th>Three point method</th>
<th>One point method</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>68</td>
<td>67</td>
<td>65</td>
<td>63</td>
</tr>
<tr>
<td>In dry season, Khon Kaen province - Treated site with recommended rate n = 63</td>
<td>3.53 ± 0.27</td>
<td>3.50 ± 0.28</td>
<td>3.39 ± 0.32</td>
<td>1.28 ± 0.35</td>
</tr>
<tr>
<td>n</td>
<td>22.3 ± 0.82</td>
<td>22.2 ± 1.03</td>
<td>22.0 ± 0.94</td>
<td>20.3 ± 2.58</td>
</tr>
<tr>
<td>- Intensive method</td>
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<tr>
<td>- Thai farmer school method</td>
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<tr>
<td>- Three point method</td>
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<td>- One point method</td>
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</tbody>
</table>

### 1) Herbivores

<table>
<thead>
<tr>
<th>Method</th>
<th>Intensive method</th>
<th>Thai farmer school method</th>
<th>Three point method</th>
<th>One point method</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>173</td>
<td>171</td>
<td>165</td>
<td>159</td>
</tr>
<tr>
<td>In dry season, Kalasin province - Treated site with double rate n = 202</td>
<td>7.43 ± 0.21</td>
<td>7.42 ± 0.21</td>
<td>7.23 ± 0.13</td>
<td>7.14 ± 0.24</td>
</tr>
<tr>
<td>n</td>
<td>19.1 ± 0.74</td>
<td>19.2 ± 0.42</td>
<td>15.0 ± 4.11</td>
<td>10.3 ± 4.57</td>
</tr>
<tr>
<td>- Intensive method</td>
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<tr>
<td>- Thai farmer school method</td>
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<tr>
<td>- Three point method</td>
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<td>- One point method</td>
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</tbody>
</table>

### 2) Predators

<table>
<thead>
<tr>
<th>Method</th>
<th>Intensive method</th>
<th>Thai farmer school method</th>
<th>Three point method</th>
<th>One point method</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>45</td>
<td>31</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td>n = 50</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>In dry season, Kalasin province - Treated site with recommended rate n = 61</td>
<td>7.39 ± 0.17</td>
<td>7.33 ± 0.16</td>
<td>7.19 ± 0.14</td>
<td>7.17 ± 0.14</td>
</tr>
<tr>
<td>n</td>
<td>14.5 ± 0.71</td>
<td>14.6 ± 0.52</td>
<td>13.9 ± 0.86</td>
<td>13.9 ± 1.45</td>
</tr>
<tr>
<td>- Intensive method</td>
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<tr>
<td>- Thai farmer school method</td>
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<td>- Three point method</td>
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<td>- One point method</td>
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</tbody>
</table>

### 3) Parasitoids

<table>
<thead>
<tr>
<th>Method</th>
<th>Intensive method</th>
<th>Thai farmer school method</th>
<th>Three point method</th>
<th>One point method</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>38</td>
<td>26</td>
<td>35</td>
<td>22</td>
</tr>
<tr>
<td>n = 61</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In dry season, Kalasin province - Treated site with recommended rate n = 31</td>
<td>7.30 ± 0.16</td>
<td>7.24 ± 0.15</td>
<td>7.36 ± 0.14</td>
<td>7.17 ± 0.24</td>
</tr>
<tr>
<td>n</td>
<td>14.4 ± 0.69</td>
<td>14.3 ± 0.67</td>
<td>14.2 ± 0.92</td>
<td>14.1 ± 1.10</td>
</tr>
<tr>
<td>- Intensive method</td>
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<tr>
<td>- Thai farmer school method</td>
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<td>- Three point method</td>
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<td>- One point method</td>
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</table>

### 4) Detritivores

<table>
<thead>
<tr>
<th>Method</th>
<th>Intensive method</th>
<th>Thai farmer school method</th>
<th>Three point method</th>
<th>One point method</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>54</td>
<td>43</td>
<td>40</td>
<td>31</td>
</tr>
<tr>
<td>n = 31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In dry season, Khon Kaen province - Treated site with recommended rate n = 31</td>
<td>3.58 ± 0.29</td>
<td>3.26 ± 0.23</td>
<td>3.11 ± 0.17</td>
<td>3.07 ± 0.21</td>
</tr>
<tr>
<td>n</td>
<td>13.5 ± 0.71</td>
<td>13.4 ± 0.52</td>
<td>13.3 ± 0.95</td>
<td>13.0 ± 1.33</td>
</tr>
<tr>
<td>- Intensive method</td>
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</tr>
<tr>
<td>- Thai farmer school method</td>
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<tr>
<td>- Three point method</td>
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<td>- One point method</td>
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</tr>
</tbody>
</table>
Figure 2. Highlight of mean differences of methods of collecting arthropods by using exp Shannon-Weiner index in rice field.
Figure 3. Highlight of mean differences of methods of collecting arthropods by using $E_{sn}$ (rarefaction) in rice field
stages on the same rice hills for randomly 3-point and 1-point methods so there was no pattern of $E_{\text{sn}}$; 2) If the farmers swept aggregated hills, the $E_{\text{sn}}$ will be high. If it is not, $E_{\text{sn}}$ will be low. Hence, it would be better if the farmers used Thai farmer school method that would sweep insect many times and in all areas to ensure that farmer would face both aggregated and contagious hills.

In terms of species richness; $E_{\text{sn}}$ (rarefaction) from previous study of Heong et al., (2005) entitled “the changes in pesticide use and arthropods biodiversity at IRRI research farm” in 1989 and 2005 following IPM policy, the arthropods were obtained by insect sampling equipment, one type of intensive methods. They showed that $E_{\text{sn}}$ and exp $H'$ increased after reducing pesticide in rice field as follow: in 1989, $E_{\text{sn}}$ of herbivores, predators, parasitoids and detritivores were 13.6, 37.6, 17.1 and 5.6., respectively. In 2005 after conducting IPM policy, $E_{\text{sn}}$ increased and were 36.0, 65.0, 38.0 and 30.0, respectively. The data of exp $H'$ in 1989 and 2005 also showed the same tendency.

Moreover, the farmers expressed their opinions after collecting insects and spiders that they concerned about the distance and steps of walking that were not stable and also worried about whether they walked straightly or not for the intensive method. Regarding Thai farmer school method, it was convenience, not expensive, and they could make friends with other farmers who had rice fields close to theirs. They could share idea when facing problems and felt happy when they had activities together. This method was easier way to collect arthropods in their community. Therefore, the farmer school method is an alternative due to reasons that it’s simpler, cheaper and more practical as compared to the intensive method.

ACKNOWLEDGEMENTS

Deep appreciation goes to the research advisory committee. We would like to thank all participants who participated in this study. especially, Dr. Kong Luen Heong from International Rice Research Institute (IRRI), Philippines, Prof. Dr. Pierre Capel, University of Utrecht in Netherland and Dr. Samart Wanchana, Postdoctoral Fellow at IRRI for their helpful comments to improve this manuscript. This work was supported in part by Ubon Ratchathani Rajabhat University, Mahasarakham University, Khon Kaen University and National Research Council of Thailand.

REFERENCES

Screening of Potential *Aspergillus* spp. for Production of Fermented Soybean with High Antioxidative Activity

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ABSTRACT

Thirty two strains of *Aspergillus* spp. were screened by their ability to increase antioxidative capacity of fermented soybean broth. Among all strains tested, soybean inoculated with *A. oryzae* BCC 3088 exhibited the highest ABTS⁺ scavenging activity, followed by those inoculated with *A. terricola* BCC 3026, *A. ornatus* BCC 3101 and *A. oryzae* BCC 3083, respectively. The similar results were observed when these strains were inoculated in solid-state fermentation of soybeans as shown by both ABTS⁺ scavenging activity and ferric reducing ability power (FRAP). Analysis of aglycone isoflavones in fermented soybeans after fermentation suggested that the proportion of aglycones in total isoflavones was highest in soybeans inoculated with *A. oryzae* BCC 3088, followed by *A. terricola* BCC 3026, *A. ornatus* BCC 3101, *A. oryzae* BCC 3083 and non-inoculated fermented soybeans, respectively. Assay of β-glucosidase activity indicated that the high β-glucosidase activity was related to the high antioxidative activity which was a culture-dependent characteristic of starter organisms. The results indicated the potential of *A. oryzae* BCC 3088 for production of fermented soybean with high antioxidative activity.

Key words: Fermented soybeans, *Aspergillus*, β-Glucosidase, Antioxidative activity, ABTS radical-scavenging activity, Ferric reducing ability power, Isoflavone substances
INTRODUCTION

Dietary antioxidants have gained much interest due to the preventive effects from free radicals that are known to be responsible for an oxidative damage to the living systems. Amongst various sources of natural antioxidants, the supplements from soybeans have been developed as a result of several naturally-occuring phenolic compounds and flavonoids, especially isoflavone (Hanasaki et al., 1994). Considerable evidences for a variety of health benefits associated with the consumption of cultured soy products have been reported (Lin and Yen, 1999; Marinova et al., 2005). Consequently, the intake of fermented soybean-derived antioxidants with free radical-neutralizing ability may be of importance for the prevention of oxidative damages and has a corresponding beneficial effect on human health (Steinberg, 1991; Jang et al., 1997). Therefore, the development of high antioxidative soybean products might play an important role in overall disease prevention and enhancement of well-being (Cassidy, 1996).

A filamentous fungus, Aspergillus oryzae, is the key organism in the production of several traditional foods. Its solid-state cultivation (SSC) has been confirmed to be the secret for the high productivity of secretory hydrolases, vital for the fermentation process and for effective degradation of raw materials. In oriental countries, Aspergillus spp. are usually inoculated into the solid culture of steamed soybean, rice or barley in koji preparation of various traditional fermented food products. In addition, fermentation with some strains of Aspergillus oryzae has been known to produce antioxidative substances. For example, the antioxidative activity of fermented soybean products such as miso inoculated with Aspergillus oryzae was significantly higher than in non-fermented steamed soybeans (Esaki et al., 1999a). Therefore, the objective of this study was to select Aspergillus strain displaying the highest ability to increase ABTS$^+$ scavenging activity and ferric reducing ability power. Besides, the amounts of potential antioxidative substances, isoflavones aglycones (daidzein and genistein), were also investigated.

MATERIALS AND METHODS

Chemicals and reagents

2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), p-nitrophenyl-β-D-glucopyranoside (pNPG), and p-nitrophenol were purchased from Wako Pure Chemical Industries, Osaka, Japan. Ferrous sulphate (FeSO$_4$) was purchased from Carlo Erba, Italy. Authentic standards of daidzin, genistin, daidzein and genistein were purchased from Sigma Chemical (St. Louis, MO, USA). HPLC-grade methanol was purchased from Fisher Scientific (UK). All other chemicals were of analytical grade.

Preparation of microorganisms

Thirty-two pure isolates of Aspergillus spp. were obtained from the BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. All these microorganisms were isolated
from fermented food products such as soy sauce, miso, koji and Japanese sake (Table 1). The freeze-dried culture was rehydrated with 1 mL of sterile distilled water. Few drops of cell suspension were inoculated onto potato dextrose agar (PDA) from Difco (Franklin Lakes, MD, USA) and incubated at 37°C for 3 days. The growing colonies were transferred to the new PDA plate and incubated at 37°C for 5 days. Spores of the fungi were harvested by flooding the surface of the agar with sterile distilled water and aseptically filtered through three layers of the sterile gauze. The turbidity of spore suspension was adjusted to 0.5 McFarland unit (Pfaller et al., 1995) and used as inoculum for the fermentation of soybeans.

Table 1. Aspergillus strains used in the screening test

<table>
<thead>
<tr>
<th>Aspergillus spp.</th>
<th>Strains</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. oryzae</td>
<td>BCC 3103</td>
<td>Soy sauce</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>BCC 3083</td>
<td>Koji</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>BCC 3373</td>
<td>Soy sauce, Miso</td>
</tr>
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<td>A. oryzae</td>
<td>BCC 3088</td>
<td>Koji</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>BCC 3087</td>
<td>Koji</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>BCC 3048</td>
<td>Fermented soybean</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>BCC 3102</td>
<td>Soy sauce</td>
</tr>
<tr>
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<td>BCC 13295</td>
<td>Koji</td>
</tr>
<tr>
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</tr>
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<td>Unknown</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>BCC 14615</td>
<td>Sake koji</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>BCC 14616</td>
<td>Koji</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>BCC 6128</td>
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<td>A. oryzae</td>
<td>BCC 7238</td>
<td>Unknown</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>BCC 7051</td>
<td>Unknown</td>
</tr>
<tr>
<td>A. sojae</td>
<td>BCC 3037</td>
<td>Unknown</td>
</tr>
<tr>
<td>A. niger</td>
<td>BCC 3344</td>
<td>Soy sauce</td>
</tr>
<tr>
<td>A. niger</td>
<td>BCC 3025</td>
<td>Koji</td>
</tr>
<tr>
<td>A. terricola</td>
<td>BCC 3026</td>
<td>Fermented soybean</td>
</tr>
<tr>
<td>A. flavas</td>
<td>BCC 3041</td>
<td>Koji</td>
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<td>Koji</td>
</tr>
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<td>A. awamori</td>
<td>BCC 13292</td>
<td>Koji</td>
</tr>
<tr>
<td>A. kawachii</td>
<td>BCC 13291</td>
<td>Unknown</td>
</tr>
<tr>
<td>A. japonicus</td>
<td>BCC 18313</td>
<td>Unknown</td>
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<tr>
<td>Aspergillus sp.</td>
<td>BCC 17548</td>
<td>Koji</td>
</tr>
<tr>
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<td>BCC 17549</td>
<td>Koji</td>
</tr>
<tr>
<td>Aspergillus sp.</td>
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<tr>
<td>Aspergillus sp.</td>
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</tr>
<tr>
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<td>BCC 17552</td>
<td>Koji</td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>BCC 17553</td>
<td>Koji</td>
</tr>
</tbody>
</table>
Preparation of soybean broth

Soybeans \textit{(Glycine max (L) Merr SJ2)} were obtained from Limsakdakun Co. Ltd. (Chiang Mai, Thailand). Whole soybeans were ground into powder by a blender (Tomex model A328, Thailand), mixed with 1 L of distilled water and then autoclaved at 121˚C for 30 min (Hirayama model HVA-85/110, Japan). After cooling at the room temperature, the supernatant of autoclaved soybean mixture was recovered by centrifugation, using a centrifuge model JE 25 (Beckman Coulter, Inc., CA, USA) at 12,000 xg at 4˚C for 15 min and referred to as soybean broth. Soybean broth (5 mL) was inoculated with 1 mL of spore suspension with concentration of 1x10^6 spores/mL and incubated at 30˚C for 4 days in an incubator shaker (Innova model 4100, Germany). The culture filtrates were centrifuged at 12,000 xg at 4˚C for 15 min. The supernatant was recovered and used for analysis.

Solid state fermentation of soybeans

Whole soybeans were washed, soaked in water for 12 h and then autoclaved at 121˚C for 30 min. After cooling, the autoclaved soybeans were inoculated with the spore suspension of selected strains of fungi at the level of 1x10^6 spores/g of cooked soybeans. The inoculated soybeans were then incubated at 30˚C and sampled at 24 h interval up to 4 days. The samples were immediately ground into powder in liquid nitrogen, using a blender (Model BBL550XL, Hawaii, USA). The samples were stored at -20˚C until use. To prepare methanol extract, powdered sample (1 g) was extracted in 5 mL of methanol with shaking at 60 rpm in a water bath for 12 h at 37˚C. The fermented soybean extracts were recovered by centrifugation at 12,000 xg at 4˚C for 15 min. The methanol extract was vacuum-concentrated at 40˚C and dried to dryness by a freeze-dryer (Labconco Corporation, USA).

ABTS\textsuperscript{+} scavenging activity assay

The ABTS radical cation (ABTS\textsuperscript{+}) scavenging activity was determined by the method of Roberta et al., (1998). ABTS\textsuperscript{+} was produced by reacting ABTS stock solution (7 mM) in distilled water with 2.45 mM potassium persulfate. The mixture was allowed to stand in the dark at the room temperature for 12-16 h before use. The ABTS\textsuperscript{+} solution was diluted with distilled water to the absorbance of 0.70-0.90 at 734 nm. In the tested reaction, fermented soybean broth, fermented soybean extract (20 µL) or standard (Trolox) were mixed with distilled water (80 µL) and 2 mL of ABTS\textsuperscript{+} working solution. After 3 min incubation at the room temperature, absorbance was then measured at 734 nm. Scavenging effect on ABTS radical ability of fermented soybean broths and fermented soybean extracts were expressed as mg Trolox/ mL sample and mg Trolox/ g fermented soybeans, respectively.
**Ferric reducing ability power (FRAP) assay**

Ferric reducing ability power (FRAP) was determined by the method of Benzie and Strain (1996). FRAP reagent was prepared by mixing 300 mM acetate buffer pH 3.6 with 10 mM TPTZ and 20 mM ferric chloride. The mixture was mixed for 15 seconds and its absorbance was recorded at 593 nm. Fermented soybean extract or standard (FeSO$_4$) solution was added in freshly prepared FRAP reagent. After 4 min of mixing, absorbance was then measured at 593 nm. The change in absorbance (Δ$A_{593 \text{ nm}} = A_{593 \text{ nm after}} - A_{593 \text{ nm before}}$) was calculated for each sample and related to Δ$A_{593 \text{ nm}}$ of Fe$^{2+}$ standard solution. The FRAP of fermented soybeans was expressed as mg FeSO$_4$/g fermented soybeans.

**β-glucosidase activity assay**

β-glucosidase activity was determined by the method of Esaki et al., (1999b) by using p-nitrophenyl-β-D-glucopyranoside (p-NPG) as a substrate. Fermented soybean powder was extracted with phosphate-citrate buffer pH 6.0 (1:5, w/v). Extraction was carried out by sonicating the mixture for 20 min at 4°C. The fermented soybean extract was recovered by centrifugation at 12,000 xg at 4°C for 15 min. The fermented soybean broth or fermented soybeans extract 0.5 mL was mixed with 2.0 mL of 1 mM p-NPG in a 0.1 M phosphate-citrate buffer (pH 6.0). The reaction mixture was incubated at 30°C for 20 min in a water bath, then stopped reaction by adding 2.5 mL of 0.5 M sodium carbonate. The resulting p-nitrophenol was immediately monitored at 420 nm. One unit of β-glucosidase was defined as the amount of enzyme which liberated 1 µmol of p-nitrophenol per min with specified condition.

The protein content of fermented soybean broths and fermented soybean extracts was determined by the method of Lowry et al., (1951), using bovine serum albumin (BSA) as a standard.

**HPLC analysis for isoflavone compositions**

In order to verify the presence of isoflavone composition, powdered sample was extracted with methanol as previously described and filtered through a 0.45 µm membrane (Millipore Co., Bedford, MA, USA) prior to analysis by HPLC (Griffith and Collison, 2001). Reversed phase HPLC analysis was carried out with Hewlett-Packard HP 1100 series equipped with an autosampler, DAD detector, and HP ChemStation Software (Scientific Equipment Source, Pickering, Canada), using a BSD Hypersil C-18 column (4.6 x 250 mm, 5 µm). For the analysis of isoflavones, the mobile phase was composed of solvent A ($H_2O$:methanol:acetic acid, 88:10:2, v/v) and solvent B (methanol:acetic acid, 98:2, v/v). Following the injection of 20 µL of sample, solvent A was increased from 90% to 100% over 20 min, and then held at 35% for 10 min. The solvent flow rate was 1 mL/min and the eluted isoflavones were detected at 254 nm. The column temperature was controlled at 25°C. Quantitative data for daidzin, daidzein, genistin, genistein, were obtained from comparison with known standards.
Statistical analysis

All experiments were run in duplicate with triplicate determinations. Analysis of variance (ANOVA) and mean comparison were performed by Duncan’s multiple range test (Steel and Torrie, 1980). Analysis was carried out using SPSS 11.0 for windows (SPSS Inc, Chicago, IL, USA). Scatter plot was performed to determine the correlation between ABTS⁺ scavenging activity and β-glucosidase activity or total aglycones. Correlation coefficients of linear regression (R²) were computed by using the Statistical Package for Social Science (SPSS for windows version 17.0: SPSS Inc.).

RESULTS AND DISCUSSION

Screening of Aspergillus strains in fermented soybean broth

Almost soybean broths inoculated with fungal inoculation exhibited stronger ABTS⁺ scavenging activity than non-inoculated soybean broth (control) (Figure 1). However, the enhanced effect on antioxidative activity of fermented soybean broths varied depending on the strains of Aspergillus strains inoculated. Amongst 32 strains tested, the fermented soybean broth inoculated with A. oryzae BCC 3088 exhibited the highest antioxidative activity (0.48 TEAC/mL sample), followed by A. terricola BCC 3026 (0.46 TEAC/mL sample), A. ornatus BCC 3101 (0.45 TEAC/mL sample) and A. oryzae BCC 3083 (0.44 TEAC/mL sample), respectively. The ABTS radical scavenging assay is one of the popular indirect methods of determining the antioxidative capacity of compounds (Roberta et al., 1998). In the absence of antioxidants, the ABTS radical is rather stable, but it reacts energetically with an H atom donor and is converted into a non-colored form of ABTS. Therefore, these 4 Aspergillus strains were selected for further study.
Figure 1. Antioxidative activity by scavenging effect on ABTS radical ability assay of fermented soybean broths with 32 strains of *Aspergillus*. Each fungal strain code represented in Table 1. A control contained only soybean broths without inoculation. The antioxidative activity of fermented soybean broths was measured at the fourth day of fermentation. Each value was the averages of 4 replicates.
Antioxidative activities of fermented cooked soybeans

The scavenging effects on ABTS\(^+\) and FRAP of cooked soybeans fermented with 4 selected *Aspergillus* strains are shown in Figures 2a and 2b, respectively. Soybeans fermented with 4 selected *Aspergillus* strains exhibited stronger antioxidative activity than soybeans without inoculation (control). The results obtained were in agreement with those previously observed in fermented soybean broth. Furthermore, the fermented soybeans incubated with *A. oryzae* BCC 3088 at the fourth day possessed the highest antioxidative activity (1.59 TEAC/g fermented soybeans) among all strains selected from the fermented soybean broth.

FRAP of fermented soybeans inoculated with 4 selected *Aspergillus* strains at different times ranged from 0.097 and 0.650 mg FeSO\(_4\)/g fermented soybeans (Figure 2b). The fermented soybeans incubated with *A. oryzae* BCC 3088 at the fourth day possessed the highest FRAP (0.65 mg FeSO\(_4\)/g fermented soybeans) among all strains selected, followed by those inoculated with *A. terricola* BCC 3026, *A. ornatus* BCC 3101 and *A. oryzae* BCC 3083, respectively. The results were correlated with ABTS\(^+\) scavenging activity of fermented soybeans. The FRAP assay is a method for assessing antioxidative activity based on reducing ferric to ferrous ion (Benzie and Strain, 1996). The samples reduced Fe\(^{3+}\)/tripyridyltriazine complex, present in stoichiometric excess, to the blue-colored ferrous complex form with an increase in absorbance at 593 nm. The results were in accordance with Santiago et al., (1992), Berghofer et al., (1998) and Chung et al., (2002) who reported that antioxidative activity of fermented soybean products could be enhanced through fermentation with certain microorganisms, especially the filamentous fungi such as *Aspergillus* and *Rhizopus*. 
Figure 2. Antioxidative activity by scavenging effect on ABTS radical cation (ABTS⁺) scavenging activity assay (A) and ferric reducing ability power assay (FRAP) (B) of fermented soybeans with 4 strains of Aspergillus during fermentation. A control contained only steamed soybeans without inoculation. Each value was the averages of 2 separate experiments.
Isoflavones content of fermented soybeans

The changes in isoflavones content of fermented soybeans inoculated with all selected fungal strains at day 0 and day 4 of fermentation are shown in Table 2. The primary isoflavones in soybeans are daidzein, genistein and their respective β-glycosides, daidzin and genistin. Almost soy products have a total isoflavone concentration of 1-3 mg/g in which the isoflavones appeared mostly as the glycoside conjugates (Góes-Favoni et al., 2010). After fermentation, aglycone concentration of soybeans fermented with A. oryzae BCC 3088 became remarkably high whereas glycoside concentration decreased significantly after 4 days of fermentation (Table 2). As for the increases of isoflavone aglycone during the fermentation, the proportion of aglycones in total isoflavones was highest in soybeans fermented with A. oryzae BCC 3088, followed by those inoculated with A. terricola BCC 3026, A. ornatus BCC 3101, A. oryzae BCC 3083 and non-inoculated fermented soybeans, respectively. In consideration of the possible free radical scavenging activity of fermented soybean, isoflavone aglycones are considered to be responsible for the overall increased antioxidant properties in both oil and lipid/aqueous systems. A higher content of aglycones might be a result from the action of β-glucosidase (β-D-glycoside glycohydrolase, EC 3.2.1.21), endogenous in soybeans (Matsura et al., 1995), and the associated β-glucosidase of the fermenting microbes (Kaya et al., 2008) which promoted the hydrolysis of the β-glucoside conjugates, converting them to aglycones.

Table 2. The changes in isoflavone glucosides (daidzin and genistin) and isoflavone aglycones (daidzein and genistein) content of soybeans at day 0 and day 4 of fermentation.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Time</th>
<th>Daidzin</th>
<th>Genistin</th>
<th>Total glucosides</th>
<th>Daidzein</th>
<th>Genistein</th>
<th>Total aglycones</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCC 3026</td>
<td>Day 0</td>
<td>1.439±0.012</td>
<td>1.397±0.001</td>
<td>2.836±0.023</td>
<td>0.081±0.023</td>
<td>0.122±0.002</td>
<td>0.230±0.002</td>
</tr>
<tr>
<td></td>
<td>Day 4</td>
<td>0.154±0.027</td>
<td>0.264±0.066</td>
<td>0.418±0.02</td>
<td>0.727±0.002</td>
<td>0.778±0.008</td>
<td>1.505±0.008</td>
</tr>
<tr>
<td>BCC 3088</td>
<td>Day 0</td>
<td>1.466±0.052</td>
<td>1.374±0.060</td>
<td>2.84±0.014</td>
<td>0.114±0.000</td>
<td>0.145±0.002</td>
<td>0.259±0.002</td>
</tr>
<tr>
<td></td>
<td>Day 4</td>
<td>0.295±0.006</td>
<td>0.463±0.006</td>
<td>1.167±0.007</td>
<td>0.918±0.000</td>
<td>2.085±0.000</td>
<td></td>
</tr>
<tr>
<td>BCC 3083</td>
<td>Day 0</td>
<td>1.290±0.027</td>
<td>1.305±0.018</td>
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<tr>
<td></td>
<td>Day 4</td>
<td>0.169±0.004</td>
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<td>0.629±0.000</td>
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<td>BCC 3101</td>
<td>Day 0</td>
<td>1.343±0.005</td>
<td>1.359±0.001</td>
<td>2.702±0.002</td>
<td>0.082±0.000</td>
<td>0.105±0.002</td>
<td>0.187±0.002</td>
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<tr>
<td></td>
<td>Day 4</td>
<td>0.124±0.006</td>
<td>0.234±0.053</td>
<td>0.358±0.005</td>
<td>0.675±0.005</td>
<td>0.741±0.002</td>
<td>1.416±0.002</td>
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<td>Control</td>
<td>Day 0</td>
<td>1.876±0.057</td>
<td>1.863±0.052</td>
<td>3.739±0.070</td>
<td>0.107±0.000</td>
<td>0.163±0.005</td>
<td>0.270±0.005</td>
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<td></td>
<td>Day 4</td>
<td>1.069±0.040</td>
<td>1.254±0.055</td>
<td>2.323±0.006</td>
<td>0.609±0.001</td>
<td>0.306±0.001</td>
<td>0.915±0.001</td>
</tr>
</tbody>
</table>

Means with different letters in the same column and row indicated significant differences (P<0.05) between treatments.
Correlation between antioxidative activity and β-glucosidase activity

β-glucosidase activity of the fermented soybean broths and cooked soybeans incubated with Aspergillus are shown in Figures 3a and 3b. The β-glucosidase activities of fermented soybean broths inoculated with all fungal strains were higher than that of the non-inoculated soybean broth (control). The fermented soybean broths incubated with A. oryzae BCC 3088 showed the highest β-glucosidase activity, followed by A. terricola BCC 3026, A. ornatus BCC 3101 and A. oryzae BCC 3083, respectively. β-Glucosidase catalyzes the hydrolytic cleavage of β-glycosidic linkages of low molecular mass glycosides and is also to be a key enzyme in the enzymatic release of aromatic compounds from glucosidic precursors found in fruits and fermented products (Gueguen et al., 1996; Christine et al., 1998). Aspergillus strains are known for their ability to produce β-glucosidase with significantly higher yields than the other species. Esaki et al., (1999a) reported that β-glucosidase produced from A. saitoi in the fermented soybean extract gradually hydrolyzed the glucoside isoflavones into aglycone isoflavones. It was suggested that the catalytic action of β-glucosidase during fermentation liberated aglycones of isoflavone glucosides, resulting in the increased antioxidative activities (Esaki et al., 1994). Hence, the higher antioxidative activities observed could thus be related to their high β-glucosidase activity.

For solid-state fermentation, the specific activity of β-glucosidase activity of fermented soybeans ranged between 0.34 and 6.87 unit/g fermented soybeans (Figure 3). The β-glucosidase activity of all fermented soybeans was higher than that of the soybeans without inoculation (control). Additionally, the fermented soybeans incubated with A. oryzae BCC 3088 at the fourth day showed the highest β-glucosidase activity. β-Glucosidase activity gradually increased with fermentation time, especially after 2 days which was the stage of sporulation. Murakami et al., (1984) reported that the β-glucosidase from filamentous fungi during the fermentation liberated the isoflavones in soybeans, resulting in the increased antioxidative activity in miso and tempeh. β-glucosidase produced from the A. saitoi fermentation gradually converted glucosides into aglycones, potential antioxidative substances (Esaki et al., 1994). Therefore, the high β-glucosidase enzyme activity in fermented soybeans caused by filamentous fungi was related to antioxidative activity as well.

In the present study, antioxidative capacity of fermented soybeans was positively correlated with total aglycone concentration ($R^2 = 0.71, P<0.05$) and β-glucosidase activity ($R^2 = 0.84, P<0.05$). These results confirmed that increased total aglycone isoflavone during fermentation mainly contributed to the enhanced antioxidative activity of soybean fermented with starter culture. Summarily, the results indicated that the fermented soybeans incubated with A. oryzae BCC 3088 gave the highest amount of isoflavone aglycones content, resulting in the highest antioxidative activities since β-glucosidase hydrolyzed daidzin and genistin and then released daidzein and genistein, the potential antioxidative substances, during fermentation. The finding in this study corresponded with Chia-Hung et al., (2006) who reported that soybean fermented with filamentous fungi containing abundance of β-glucosidase enzyme possessed enhanced antioxidative activities in various model systems.
Figure 3. β-Glucosidase activity of fermented soybean broths measured at the fourth day of fermentation (A) and in fermented soybean during fermentation (B) with 4 selected strains of *Aspergillus*. A control contained only soybean broths without inoculation. β-glucosidase activity of fermented soybean broths was measured at the fourth day of fermentation. Each value was the averages of 4 replicates.
CONCLUSION

*A. oryzae* BCC 3088 had the most potential antioxidative activities among the 32 strains of *Aspergillus* tested and possessed enhanced ABTS$^+$-scavenging effect, FRAP and higher amount of potential antioxidative substances, isoflavone aglycones (daidzein and genistein). Our results also suggested the possibility to enhance antioxidative activity of fermented soybeans by using *Aspergillus* strain that was capable of producing β-glucosidase. The fermented soybeans inoculated with *Aspergillus* showed higher β-glucosidase enzyme and isoflavones content in comparison with those of control, indicating the higher antioxidative activities. However, the mechanism and the essential biofactors contributing to the antioxidative activity remain to be further clarified.

![Figure 4. Scatter plot depicting correlation between scavenging effects on ABTS$^+$ with β-Glucosidase activity (▲) and total aglycone concentration (●).](image-url)
ACKNOWLEDGEMENTS

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REFERENCES


Development of an Instrument for Measuring Attitudes towards Safe Motorcycle Driving Behaviors among Thai Adolescents

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ABSTRACT

This study was a part of the main study using an ecological context to develop instruments to measure factors influencing safe motorcycle driving behaviors among Thai adolescents. The study aimed to develop an instrument to specifically measure Thai adolescents’ attitudes towards these behaviors. The participants were purposefully recruited from students studying in vocational certificate levels 1-3 from vocational and technical colleges in Chiang Mai, Thailand. The instrument development research design included two phases.

The first phase was scale construction. The relevant terms and concepts were clarified and defined based on a literature review. Thirty six items with a 4-point Likert-type scale were generated using data obtained from focus group discussions and the literature. Content validity was assured by six experts. The items’ CVI ranged between .83 to 1.00 and the scale’s CVI was .90. Then the instrument was critiqued by six students to ensure clarity and readability. After the students’ review, the revised scale consisted of 35 items with 6-point Likert-type scale.

The second phase was a psychometric properties evaluation using 491 students. The construct validity was evaluated with exploratory factor analysis. Six dimensions were extracted from 25 items. The total explained variance was 58.94%. The scale’s alpha coefficient was .89 and for each dimension the coefficient ranged between .72 and .83. These results indicate that the scale is valid and reliable and can be used to assess attitudes towards safe motorcycle driving behaviors among Thai adolescents.

Key words: Scale development, Attitude, Adolescent, Motorcycle driving behavior
INTRODUCTION

Motorcycle accidents account for about 80% of all road traffic accidents in Thailand (Kanchanasut, 2004; The Royal Thai Police, 2005). Each year, approximately 200,000 of the Thai population have motorcycle accidents. On average, 18 victims are killed every day by these serious accidents (National Health Foundation, 2007). Furthermore, the number of motorcycle accidents rises every year. The majority of motorcycle accidents occur among individuals aged 15-24 years and are typically males (Bohning and Na Ayutha, 1997; Klein, 2001; National Statistic Office, 2005). In fact, males have four to five as many of deaths from motorcycle accidents compared to females throughout their life span (Klein, 2001). Driving behaviors found to be associated with traffic accidents were driving at inappropriate or excessive speeds, use of alcohol and failure to use safety devices such as helmets (Suriyawongpaisal and Kanchanasut, 2003; The Royal Thai Police, 2004; Don’t Drive Drunk Foundation, 2005; Narenthorn Center, 2005; Tanaboriboon and Satiennam, 2005).

The high incidence of motorcycle accidents is also associated with both environmental and human factors. Environmental factors include road conditions, traffic management, traffic policy, traffic law and law enforcement (Suriyawongpaisal et al., 2002; World Health Organization, 2004; Arrive Alive, 2005). Human factors were contributing elements in 95% of the accidents; particularly, unsafe driving behavior was a common element identified in those accidents (Ulleberg and Rundmo, 2003; Thai Health Promotion Foundation, 2007). Besides other factors such as age, gender, knowledge, parental and peer influence, drivers’ attitudes were found to alter adolescents’ driving behaviors (Reeder et al., 1992; Parker, Lajunen et al., 1998; Parker and Stradling, 2001; Coltheart, 2002; Laapotti et al., 2003; Ulleberg and Rundmo, 2003; Redshaw, 2004; Warner and Aberg, 2006). Lastly, adolescent tendency to thrill seek and be overconfident were also associated with traffic accidents (Kasantikul et al., 2005).

Based on the literature review, the significance and complexity of factors that influence motorcycle driving behaviors such as attitudes should be examined in order to better understand the contributing factors of safe motorcycle driving behaviors. After these factors are determined then programs for motorcycle accident prevention can be developed. However, these factors inclusive of attitude cannot be studied because of the lack of pre-existing, valid and reliable measurement tools. A significant portion of this study’s effort was directed to the development of instruments for measuring safe motorcycle driving behaviors and factors influencing safe motorcycle driving behaviors among Thai adolescents within an ecological context.

OBJECTIVES

The study aimed to develop an instrument to measure Thai adolescents’ attitudes towards safe motorcycle driving behaviors and to conduct psychometric evaluation of the newly-developed instrument. These driving behaviors included
obeying traffic laws and regulations, not drinking and driving and wearing a proper helmet while driving a motorcycle.

METHODS

Study settings

This research proposal was approved by the Research Ethics Review Committee of the Faculty of Nursing, Chiang Mai University. In addition, permission from each of the directors of the selected schools were obtained prior to the sample recruitment process. Eligible subjects were asked to voluntarily participate in the study. Participants who agreed to participate were informed and assured that the data would be kept strictly confidential and reported anonymously. Informed consent was obtained from adolescent subjects who reached the age of 18 and over. In addition, parental consent and adolescent’s assent were obtained when subjects were under 18 years of age.

Participants

The population of this study were Thai adolescents who drive a motorcycle. The settings were two vocational and technical colleges with a male majority of students in Muang District, Chiang Mai, Thailand. A purposive sampling method was employed to recruit eligible subjects who met the following inclusion criteria: (1) being male or female age 15-24 years (middle-late adolescence), (2) use of a motorcycle as the primary means of transportation in daily life and (3) willingness to participate in this study. During the data collection process, three sample groups participated in this study. The first group was assembled for the purpose of clarifying and defining concepts and to generate an item pool. Forty-four students were purposefully selected to participate in six focus group discussions. The second group was assembled for the process of reviewing the draft questionnaires for clarity and readability. In total, six students, two students of each educational level were purposefully selected. In the third group, 491 students were recruited to evaluate the psychometric properties of the instrument. Purposive sampling and stratified random sampling methods were used to recruit participants from each college. A whole class of students in each level was randomly selected from all three levels. The characteristics of the first and third groups of students are presented in Table 1.
Table 1. Characteristics of the first and the third groups’ participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>First group (n=44)</th>
<th>Third group (n=491)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number %</td>
<td>Number %</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>34 77.27</td>
<td>380 77.39</td>
</tr>
<tr>
<td>Female</td>
<td>10 22.73</td>
<td>111 22.61</td>
</tr>
<tr>
<td>Age (year)</td>
<td>Range=15-20, Mean=16.68, SD=1.05</td>
<td>Range=15-22, Mean=17.80, SD=1.03</td>
</tr>
<tr>
<td>15</td>
<td>5 11.35</td>
<td>1 0.21</td>
</tr>
<tr>
<td>16</td>
<td>15 34.10</td>
<td>46 9.37</td>
</tr>
<tr>
<td>17</td>
<td>15 34.10</td>
<td>151 30.75</td>
</tr>
<tr>
<td>18</td>
<td>8 18.18</td>
<td>152 30.96</td>
</tr>
<tr>
<td>19</td>
<td>-</td>
<td>123 25.05</td>
</tr>
<tr>
<td>≥ 20</td>
<td>1 2.27</td>
<td>13 2.64</td>
</tr>
<tr>
<td>Declined to submit age</td>
<td>-</td>
<td>5 1.02</td>
</tr>
<tr>
<td>Level of education: vocational certificate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 1</td>
<td>17 38.64</td>
<td>165 33.60</td>
</tr>
<tr>
<td>Level 2</td>
<td>13 29.54</td>
<td>156 31.77</td>
</tr>
<tr>
<td>Level 3</td>
<td>14 31.82</td>
<td>170 34.62</td>
</tr>
<tr>
<td>Frequency of motorcycle driving (day/week)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>-</td>
<td>21 4.27</td>
</tr>
<tr>
<td>3-4</td>
<td>-</td>
<td>25 5.07</td>
</tr>
<tr>
<td>5-6</td>
<td>5 11.36</td>
<td>63 12.83</td>
</tr>
<tr>
<td>7</td>
<td>39 88.64</td>
<td>370 75.37</td>
</tr>
<tr>
<td>Declined to submit information</td>
<td>-</td>
<td>12 2.44</td>
</tr>
<tr>
<td>Purpose of driving motorcycle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commuting to and from school</td>
<td>41 93.18</td>
<td>385 78.41</td>
</tr>
<tr>
<td>Other</td>
<td>3 6.82</td>
<td>106 21.59</td>
</tr>
<tr>
<td>Distance driven on a motorcycle (kilometer/day)</td>
<td>Range=3-100, Mean=30, SD=20.26</td>
<td>Range=1-110, Mean=26.30, SD=20.90</td>
</tr>
<tr>
<td>≤ 10</td>
<td>8 18.18</td>
<td>159 32.38</td>
</tr>
<tr>
<td>11-30</td>
<td>21 47.73</td>
<td>170 34.62</td>
</tr>
<tr>
<td>31-50</td>
<td>10 22.73</td>
<td>95 19.35</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>5 11.36</td>
<td>49 9.98</td>
</tr>
<tr>
<td>Declined to submit information</td>
<td>-</td>
<td>18 3.67</td>
</tr>
<tr>
<td>Role while motorcycle riding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Driver</td>
<td>37 84.10</td>
<td>384 78.21</td>
</tr>
<tr>
<td>Passenger</td>
<td>7 15.90</td>
<td>107 21.79</td>
</tr>
<tr>
<td>Own a motorcycle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>43 97.70</td>
<td>468 95.32</td>
</tr>
<tr>
<td>No</td>
<td>1 2.30</td>
<td>23 4.68</td>
</tr>
<tr>
<td>Possess a driver’s license</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22 50.00</td>
<td>297 60.49</td>
</tr>
<tr>
<td>No</td>
<td>22 50.00</td>
<td>194 39.51</td>
</tr>
<tr>
<td>Have a helmet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>39 88.64</td>
<td>456 92.87</td>
</tr>
<tr>
<td>No</td>
<td>5 11.36</td>
<td>35 7.13</td>
</tr>
<tr>
<td>Have been arrested/fined</td>
<td>(n=31)</td>
<td>(n=313)</td>
</tr>
<tr>
<td>Yes</td>
<td>31 70.45</td>
<td>313 63.75</td>
</tr>
<tr>
<td>No</td>
<td>13 29.55</td>
<td>178 36.25</td>
</tr>
<tr>
<td>Cause for being arrested/fined*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not wearing a helmet</td>
<td>18 58.07</td>
<td>270 86.26</td>
</tr>
<tr>
<td>Not possessing a valid license</td>
<td>7 22.58</td>
<td>67 21.41</td>
</tr>
<tr>
<td>Traffic violation</td>
<td>2 6.45</td>
<td>32 10.22</td>
</tr>
<tr>
<td>Others</td>
<td>4 12.90</td>
<td>26 8.31</td>
</tr>
<tr>
<td>Involved in a motorcycle accident previously</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>38 86.36</td>
<td>332 67.62</td>
</tr>
<tr>
<td>No</td>
<td>6 13.64</td>
<td>159 32.38</td>
</tr>
</tbody>
</table>

*Some students were arrested with multiple causes.
Scale Development Procedures

To develop the scale, a methodological design was employed. The scale development consisted of two phases: 1) the construction of the initial scale and 2) the evaluation of its psychometric properties.

**Phase I:** Construction of the initial scale. Both qualitative and quantitative methods were initiated in steps 1-4. Step 1: Clarify and define the concept. Data obtained from the literature review were used to develop guidelines to define the scope and organization of the concepts and the terms of the study. Step 2: Generating an item pool. The original draft of the instruments was generated in the Thai language. The data obtained from relevant literature reviews and six focus group discussions were analyzed and categorized by using a content analysis procedure. Then, items were clustered into three subscales measuring three specific behaviors that include obeying traffic law and regulations, not drinking and driving, and wearing a proper helmet. Step 3, a panel of six experts reviewed the first draft of instruments for content validity. The experts consisted of three nursing professors who are experts in the area of adolescent behaviors and experienced in instrument development, a psychology professor and expert in adolescent motorcycle driving behaviors, a pediatrician who is a specialist in child injury prevention and the school psychologist and teacher at a vocational college. Information provided by experts was used to determine an individual item’s content validity index (I-CVI) and the scale’s content validity index (S-CVI) (Polit and Beck, 2006). Then, in Step 4, six students were asked to complete the scale and evaluate its items for clarity, ease of understanding and length appropriateness of the overall questionnaire.

**Phase II:** Evaluation of psychometric properties of the instrument. A quantitative approach was employed for Step 5. In this step, field testing for item analysis, construct validity and internal consistency reliability was conducted with 491 students who volunteered to participate in this step. The package of questionnaires including a cover letter from the researcher, a consent form, the Demographic Data Collection Form and the newly-developed questionnaire was distributed to the students by the researcher and three trained assistants. After completing all questionnaires, these students were told to return them to the researcher or assistants who were present while the remaining students continued to complete their questionnaires. Those questionnaires that were returned incomplete, the researcher or the research assistants asked the students to complete them. Despite those efforts, some returned questionnaires were not completed in their entirety. Consequently, the number of returned, full completed questionnaires was 453 (92.26%).

Item analysis was performed to determine the characteristics of each item with respect to the entire scale. Each item was examined for three characteristics: descriptive statistic of items, discrimination power of items and item correlation. The criteria for retaining an item within the entire scale for this study were (1) inter-item correlation value between .30 and .70, (2) item- subscale correlation value of .50 or over, (3) an item-total correlation value above .30 (Nunnally, 1978) and (4) no substantial change of Cronbach’s alpha when an item was removed
Factor analysis was employed to examine the internal construct validity of the scale and to cluster interrelated items. Prior to performing factor analysis, the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett’s test of sphericity were applied to determine appropriateness for factor analysis. Exploratory factor analysis was performed with two different methods, then the method that resulted in the clearest, most† unambiguous response was selected.† The criteria for determining the best factor solution of factor extractions were (1) a factor with an eigenvalue of 1 or above, (2) an item with a factor loading cutoff point of .30 or greater, (3) no or few cross-loading or secondary loading items and (4) no factor with fewer than three items (Burns and Grove, 2001; DeVellis, 2003; Waltz et al., 2005).

RESULTS AND DISCUSSION

Construction of the Attitudes towards Motorcycle Driving Questionnaire (AMDQ)

Clarify and define concept. As shown in Table 2, the scope of each term was defined based on the literature review.

Table 2. Scope and definition of terms or concepts

<table>
<thead>
<tr>
<th>Term</th>
<th>Scope and Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safe motorcycle driving behaviors</td>
<td>An individual’s actions in driving a motorcycle safely which are composed of three crucial behaviors including obeying traffic laws and regulations, not drinking and driving and wearing a proper helmet while driving a motorcycle.</td>
</tr>
<tr>
<td>Obeying traffic laws and regulations</td>
<td>Driving a motorcycle while following traffic laws and regulations in terms of driving with a valid license, driving within speed limits, driving with safe passing of other vehicles, e.g., checking roads, signaling lights and obeying rules and signs as well as avoiding distractions such as cell phone use.</td>
</tr>
<tr>
<td>Not drinking and driving</td>
<td>Delaying driving a motorcycle for one hour per alcohol beverage consumed.</td>
</tr>
<tr>
<td>Wearing a proper helmet</td>
<td>Wearing a helmet that meets safety standards, fits well and fastened properly whenever operating a motorcycle.</td>
</tr>
<tr>
<td>Attitude towards safe motorcycle driving behaviors</td>
<td>One’s own evaluative assessment of safe motorcycle driving behaviors in relation to obeying traffic laws and regulations, not drinking and driving and wearing a proper helmet with some degree of approving or disapproving, valuing it as positive or negative, liked or disliked.</td>
</tr>
</tbody>
</table>
Generating an item pool. The original draft of the instrument was generated in the Thai language. The data obtained from relevant literature reviews and focus group discussions were analyzed and categorized using a content analysis procedure. The first draft of the instruments consisted of a total of 36 items. In addition, a 4-point Likert-type scale to denote degree of agreement was assigned as a response format of the AMDQ. The response statements ranged from strongly disagree to strongly agree.

Reviewing items by experts. The AMDQ with 36 items was submitted to the experts. All items were evaluated as mostly relevant or absolutely relevant to the concept. However, one item of wearing a proper helmet subscale (Athel2: “Wearing a helmet while driving a motorbike does not help decrease a severity of injury”) was suggested for deletion because of its redundancy. Therefore, 35 items remained with 15, 7 and 13 items of obeying traffic law and regulations, not drinking and driving and wearing a proper helmet subscales, respectively. The values of I-CVI of the remaining 35-items ranged from 0.83 to 1.00 and the S-CVI was 0.90. Furthermore, based on the expert’s suggestions, the response choices were expanded from four to six response categories.

Determining the clarity and readability of the questionnaire. The instructions for answering the questionnaires were evaluated as unambiguous by the second group students. The formatting of the AMDQ was easy to understand for all students. Therefore, the scale’s text†remained unchanged from the draft.

Psychometric Properties of the Scale

Results of item analysis. Discrimination power of items of this scale was investigated by using the split group response method. The item mean scores of the low score group (114 students) were compared with those of the high score group (115 students). This finding revealed that the t-values ranged from 4.34 to 17.52. All items were significant at a p value of .001. This revealed that the low score group responded to all items of the scale differently from the high score group. Therefore, all 35 items were good discriminators and appropriate to be retained in the scale.

Based on the results of item intercorrelations which consisted of inter-item correlation, item-subscale correlation, corrected item-total correlation, coefficient alpha if an item was deleted, subscale-subscale correlation and subscale-total correlation procedures, eight items (Atreg1, Atreg2, Atreg12, Atdd16, Athel23, Athel24, Athel25 and Atreg26) were deleted. Therefore, the AMDQ scale was comprised of 27 items with an overall Cronbach’s alpha of .90 and .84, .79 and .83, for the three subscales.

Results of exploratory factor analysis. Exploratory factor analysis was employed to examine the construct validity of the scale and to cluster interrelated items. The principal components analysis with varimax rotation method was performed first. The findings indicated that six dimensions were extracted and all 27 items remained. All 27 items had factor loading ranging from .30 to .84, in which seven items (Atreg5, Atreg9, Atreg14, Atdd22, Athel29, Athel30 and Athel31) loaded on two components. The picture of factor loading on each
component was unclear; nearly 25% of items did not single load in a specific component. The principal component with direct oblimin was then conducted. The results illustrated a similar picture to the first method with six components extracted. Among all 27 items, 26 items (except Atreg5) retrieved with factor loading ranging from .33 to .87 without any item loaded on two components. Based on the result of the first-order factor analysis, the principal components with direct oblimin method provided a clearer and more stable picture of factor loading. Therefore, this method was chosen for further factor analysis since it provided the best opportunity to interpret the factor solution unambiguously.

The second-order factor analysis was applied to the 26 remaining items. The processes were duplicated as they were performed during the first-order analysis. The result of the second-order factor analysis with the principal components analysis with direct oblimin rotation method revealed that all 26 items remained in six components with eigenvalues ranging from 1.03 to 7.18 and accounted for 3.98% to 27.61% of variance. All six components together explained 58.31% of variance. However, among 26 items, Atreg14 (“Violating traffic regulations while driving a motorbike is enjoyable and exiting.”) loaded on two factors. This item was not specific to any driving behavior and it was difficult to determine on what component the item should be retained. Consequently, this item was considered for deletion and was ultimately deleted. Therefore, 25 items remained to undergo a third-order factor analysis with a similar process as the first-and second-order factor analyses.

The results of the third-order factor analysis, utilizing principal component analysis with direct oblimin rotation method, showed that all 25 items remained in six components with eigenvalues ranging from 1.03 to 6.83 and accounted for 4.12% to 27.32% of variance. The items that clustered under each of six dimensions with the corresponding item statements, their factor loadings and Cronbach’s alpha of each dimension and the overall scale for the final scale of the AMDQ are shown in Table 3.
Table 3. Dimension associations and item statement of factor analysis of the AMDQ

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item Statement</th>
<th>Factor Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dimension 1: Attitude towards drunk driving and driving while using a mobile phone</td>
<td></td>
</tr>
<tr>
<td>Atreg15</td>
<td>Using a mobile phone while driving does not adversely affect one’s concentration while driving.</td>
<td>0.35</td>
</tr>
<tr>
<td>Atdd17</td>
<td>Riding a motorbike while drunk is a challenge to one’s driving ability.</td>
<td>0.69</td>
</tr>
<tr>
<td>Atdd18</td>
<td>Driving while drunk is more fun than driving sober.</td>
<td>0.76</td>
</tr>
<tr>
<td>Atdd19</td>
<td>A person that doesn’t ride drunk is a coward.</td>
<td>0.59</td>
</tr>
<tr>
<td>Atdd20</td>
<td>Riding a motorbike while drunk increases my alertness compared to riding while sober.</td>
<td>0.77</td>
</tr>
<tr>
<td>Atdd21</td>
<td>When I am drunk I ride a motorbike more carefully than when I ride sober.</td>
<td>0.69</td>
</tr>
<tr>
<td>Atdd22</td>
<td>Drinking alcohol has no effect on motorcycle driving ability.</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Eigenvalue = 6.83  
Percent of variance = 27.32  
Cronbach’s alpha coefficient = 0.79

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item Statement</th>
<th>Factor Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athel31</td>
<td>Wearing a helmet while driving a motorbike makes a driver feel drowsy.</td>
<td>0.44</td>
</tr>
<tr>
<td>Athel32</td>
<td>Wearing a helmet while driving a motorbike messes up a driver’s hair.</td>
<td>0.70</td>
</tr>
<tr>
<td>Athel33</td>
<td>Wearing a helmet while driving a motorbike decreases a driver’s ability to hear.</td>
<td>0.86</td>
</tr>
<tr>
<td>Athel34</td>
<td>Wearing a helmet while driving a motorbike impairs one’s vision.</td>
<td>0.82</td>
</tr>
<tr>
<td>Athel35</td>
<td>Wearing a helmet while driving a motorbike causes a burden to the driver because there is no convenient way to secure it at each destination.</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Eigenvalue = 2.43  
Percent of variance = 9.70  
Cronbach’s alpha coefficient = 0.76

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item Statement</th>
<th>Factor Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atreg3</td>
<td>Driving a motorbike within the speed limit causes delays and lateness for school and appointments.</td>
<td>0.86</td>
</tr>
<tr>
<td>Atreg4</td>
<td>Continuing to drive a motorbike within the speed limit causes unnecessary and prolonged exposure to the sun, rain and wind.</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Eigenvalue = 1.90  
Percent of variance = 7.61  
Cronbach’s alpha coefficient = 0.72
Table 3. (Continued)

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item Statement</th>
<th>Factor Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimension 4: Attitude towards helmet use 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Athel27</td>
<td>Putting on a helmet or taking off a helmet wastes too much time.</td>
<td>-0.81</td>
</tr>
<tr>
<td>Athel28</td>
<td>Wearing a helmet just to drive a motorbike is a waste of money.</td>
<td>-0.81</td>
</tr>
<tr>
<td>Athel29</td>
<td>Wearing a helmet while driving a motorbike is cumbersome.</td>
<td>-0.71</td>
</tr>
<tr>
<td>Athel30</td>
<td>Wearing a helmet while driving a motorbike is uncomfortable.</td>
<td>-0.64</td>
</tr>
</tbody>
</table>

Eigenvalue = 1.34  
Percent of variance = 5.35  
Cronbach's alpha coefficient = 0.83

Dimension 5: Attitude towards riding in the opposite direction

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item Statement</th>
<th>Factor Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atreg10</td>
<td>Riding a motorbike against a one-way street gets me where I want to be faster.</td>
<td>-0.77</td>
</tr>
<tr>
<td>Atreg11</td>
<td>Riding a motorbike against a one-way street helps conserve gas.</td>
<td>-0.87</td>
</tr>
<tr>
<td>Atreg13</td>
<td>Riding a motorbike against a one-way street helps evade the police at the check point.</td>
<td>-0.55</td>
</tr>
</tbody>
</table>

Eigenvalue = 4.78  
Percent of variance = 1.20  
Cronbach's alpha coefficient = 0.76

Dimension 6: Attitude towards obedience of traffic light and lane

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item Statement</th>
<th>Factor Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atreg6</td>
<td>Stopping at a red light is a waste of time.</td>
<td>-0.79</td>
</tr>
<tr>
<td>Atreg7</td>
<td>Stopping at a red light wastes fuel.</td>
<td>-0.78</td>
</tr>
<tr>
<td>Atreg8</td>
<td>Continuing to ride in the motorbike lane or the left side of the road forces the rider to unnecessarily reduce speed and wastes time.</td>
<td>-0.59</td>
</tr>
<tr>
<td>Atreg9</td>
<td>Continuing to ride a motorbike on the left side of the road increases the chance of a crash.</td>
<td>-0.52</td>
</tr>
</tbody>
</table>

Eigenvalue = 1.03  
Percent of variance = 4.12  
Cronbach’s alpha coefficient = 0.73

Reliability of the AMDQ. The reliability of the AMDQ was evaluated with the Cronbach’s alpha coefficient; the result for the entire scale was .89 and for each of the dimensions the coefficient values ranged between .72 and .83. These values surpassed the expected value (.70) for a newly-developed instrument (Hair et al., 1998; Burn and Grove, 2001). Reliability also indicates high internal consistency of the scale (Polit and Beck, 2004). This high internal consistency indicates that items of the AMDQ consistently measure the same construct and show high inter-correlation (Hair et al., 1998). These results indicate that this scale is particularly a reliable scale to assess attitudes towards safe motorcycle driving among Thai adolescent motorcyclists.
CONCLUSION AND IMPLICATIONS

The AMDQ was developed as a multidimensional scale that could effectively measure affective, cognitive and behavioral aspects of attitudes towards safe motorcycle driving behaviors. A 6-point Likert-type scale was used as the outcome space or format for this scale. The overall attitude score is the sum of scores from all six dimensions. In this aspect, all retained items have a negative connotation as it relates to safe motorcycle driving behaviors and, therefore, would need to be recoded before calculating the overall score. The total scale score of attitudes could range from 25 to 150. A higher score represents attitudes in favor of safe motorcycle driving behaviors. A lower score in any single dimension indicates a negative attitude against that particular driving behavior and reflects the need for improvement through education and training.

The newly-developed scale for measuring attitudes towards safe motorcycle driving provides a number of useful findings to expand the body of knowledge for nursing research and other related fields. The AMDQ with the acceptable reliability and validity will provide research tools for hypothesis testing studies, particularly those studies which aimed to investigate to what degree a subject’s attitude contributes to safe motorcycle driving behaviors. Understanding adolescents’ attitude towards driving behaviors is important when developing intervention programs for adolescents and working with them in settings such as schools.

LIMITATIONS

Limitations of this study are related to data collection and the research instrument itself. First, some of the participant samples drawn from the 44 participants in Step 2 of focus group discussions are recruited again†to participate in Step 5 of field testing. Second, the cross-sectional design of this study is a limitation for testing predictive criterion-related validity of this newly-developed scale. Lastly, this study could not test for concurrent or predictive criterion-related validity since there are no existing scales for comparison.

RECOMMENDATIONS

Although this newly-developed scale was proven to be valid and reliable, and can be used as a research tool for assessing attitudes towards safe motorcycle driving behaviors among Thai adolescents, it still needs further research that attempts to replicate this study’s results, and further evaluates its psychometric properties. Furthermore, this newly-developed scale needs to be tested with adolescents in other settings and areas so that standardized scales can be developed and be appropriately used among Thai adolescents. In addition, a normative reference for this scale should be identified to facilitate the interpretation of the raw scores. The predictive or concurrent criterion-related validity should be tested with existing standardized scales as well as other psychometric properties such as efficiency or sensitivity of the scale should be tested in further research. Since
the sample reflected a mostly male-population, future researchers who intend to use this instrument should be aware that this scale was developed based on the perspectives of Thai male adolescents, and be cognizant of this possible limitation.

ACKNOWLEDGEMENTS

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REFERENCES


Formulation of *Houttuynia cordata* Standardized Extract Tablets

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**ABSTRACT**

The preparation and quality control of standardized *Houttuynia cordata* (HC) extract, and the formulation of tablets containing HC extract (HCE) as a food supplement product were studied. HCE was obtained by repeated maceration of dry HC powder (HCP) with ethanol and solvent removal under reduced pressure. Standardization of HCE was carried out using chromatographic methods. HCE was adsorbed onto corn starch or silicified microcrystalline cellulose 90 (SMCC90) to obtain HCE powder (HCEP), which was formulated into tablets. Quality control (QC) of HCEP and tablets was performed after preparation and after stability tests for 3 months at room temperature (RT) and at 45°C. The yield of HCE was 5.85% dried weight (DW). Chromatographic analyses showed quercetin and rutin as major components and potential QC markers. Appearances and moisture analyses of HCEP indicated SMCC90 as the superior adsorbent. The optimum formulations, with 5.29 and 3.30% Loss on drying (LOD), and repose angles of 46.2±4.8° and 28.1±2.6° for HCEP and bulk, respectively, were selected. Weight of tablets was 250±18.75 mg while hardness, friability and disintegration time were 41.5±3.2 N, 0.04% and 1.23 min, respectively. Stability test revealed that moistures of HCEP and bulks kept at RT were lower than those stored at 45°C. Tablet hardness decreased after storage at 45°C. All tablets passed friability tests, while the disintegration times were between 1-2 min. These results suggested that standardized HCE can be employed in the formulation of food supplement tablets with good uniformity and stability.

**Key words:** *Houttuynia cordata*, Standardized extract, Chromatography, Food supplement, Tablet formulation
INTRODUCTION

Houttuynia cordata Thunb. (Saururaceae) (HC) is an important traditional medicine of East and South East Asia, especially China, Japan and Thailand, where the plant is known as Kao-Tong or Plu-Kao (Bansiddhi et al., 2003). HC was reported to contain several groups of phytochemicals. Among these, flavonoids – a group of phenolic compounds that occur in both glycosidic and aglycone forms – are of importance as they are found to possess many pharmacological properties (Matsui et al., 2005; Formica and Regelson, 1995). Major flavonoids of HC were rutin and quercetin (Havsteen, 2002). Rutin was reported to treat and prevent severe acute respiratory syndrome (SARS) (Zhang and Chen, 2008) while quercetin was found to inhibit tumor growth via blockage of protein kinase activity and lactate transport (Formica and Regelson, 1995).

Literature reviews on pharmaceutical preparation of HC revealed the use of this herb in many types of products, including as food supplement, drug, beverage and cosmetics. HC was prepared in the form of injection (Lau et al., 2008; Lu et al., 2006). HC was also combined with other herbal plants in capsule dosage form (Li, 2003) and injection (Yu, 2007). Extracts of HC and other medicinal plants were prepared as buccal tablet for acute and chronic pharyngitis and stomatitis (Xuan and Lui, 2004). In Thailand, the Government Pharmaceutical Organization (GPO) manufactures a capsule formulation containing HC and other plant extracts for use as food supplement to improve immune response (Sriwanthana et al., 2007). Preparations of sole HC are commercialized in the forms of powdered plant capsule, fermented drinks and wines. The form of tablet, both of HCP or HCE, has not been found.

Tablet is generally the most desirable dosage form as it has advantages over other forms in terms of consistency and accuracy of active compound(s) for a unit dose, tampered-proof, low cost, convenience of taking and carrying and storage. However, several reports suggested that rheological property and compressibility were two main obstacles for tabletting of plant extract. Proposed solutions to overcome these problems included the wet granulation, the preparation of spray-dried extract powder, or the use of pharmaceutical excipients to enable direct compression (Plazier-Vercamen and Bruwier, 1986; Díaz et al., 1996; Renoux et al., 1996; Palma et al., 2002). Tablets of plant extract contained higher amounts of active components than those in capsules of herbal powder. In addition, herbal extract formulation minimized the rate of microbial contamination often associated with the herbal powder preparation. The quality control of active compounds can also be facilitated in the extract formulation which leads to the higher quality and safety of medicinal plant products for modern medicines. This study reports the preparation and quality control of standardized HCE and the formulation of stable tablets containing consistent HCE as a food supplement product.
MATERIALS AND METHODS

Materials
All chemicals used in the preparation and analysis of HCE were of analytical grade or equivalent, except those used in high performance liquid chromatography (HPLC) were of HPLC grade. Standard rutin and quercetin were purchased from Fluka, Germany. SMCC90 was from JRS Pharma LP., Germany (batch no. P9D7D55). Corn starch was the product of Roquette, France (lot no. 3959 0094). Other pharmaceutical excipients were of pharmaceutical grade. GPO’s NaturePlex® capsules were purchased from a local pharmacy store.

Preparation of HC plant materials
Fresh aerial parts of 3-month grown HC, from which the leaves, branches and posy were reportedly contained flavonoid-glycosides (Eui et al., 1996; Bansiddhi et al., 2003), were obtained from Sun-Phi-Sua sub-district, San-Sai district, Chiang Mai in August-October 2008. The identity of the plant was authenticated and a herbarium voucher specimen was prepared and deposited at the Faculty of Pharmacy, Chiang Mai University. Plant materials were dried at 50˚C for 3 days in a hot air oven. Dried HC was milled with cutting mill and was sieved through mesh no.40 to obtain HCP. The moisture content (% LOD) of the dried powder was determined.

Extraction of HCP
HCP was macerated with ethanol (Kim et al., 2007) at a ratio of HCP: 95% ethanol as 1:10 for 20 h (3 times), then filtered through the filter paper (Whatman no.1). The filtrate was collected, combined and evaporated under vacuum at 50˚C via a rotary evaporator to obtain HC extract (HCE) (Zhang and Chen, 2008). HCE was weighed to calculate yield percentage, the weight of tablet and the concentration for quality control.

Preparation of HCE for analysis
HCE was fractionated with liquid-liquid partition technique into four portions: hexane (F1), chloroform (F2), ethyl acetate (F3) and water (F4). Each fraction was evaporated to dryness and reconstituted in methanol.

Identification of Terpenes and Flavonoids in HCE
HCE and F1-F4 were analyzed using thin-layer chromatography (TLC) with rutin and quercetin as standards. The evaluation was carried out under visible light (VL) and ultraviolet light (UV) at 254 and 365 nm. Silica gel GF 254 Aluminum sheets and mixed solvents were used as stationary phase and mobile phase, respectively, with 15 cm solvent front Geraniol (0.2%v/v in methanol) was used as a standard in terpene test, with petroleum ether: ethyl acetate: formic acid ratio of 47:2:1 as developing solvent (DVS). Vanillin-phosphoric acid was used as spraying reagent and the sheets were heated at 120˚C for 10 min before the chromatograms was observed. For flavonoid tests, rutin and quercetin were used as standards. DVS was the upper fraction of diethyl ether: formic
acid: water (90:20:30) mixture (Wagner et al., 1990; Bansiddhi et al., 2003). The chromatogram was observed after 1% aluminum chloride in ethanol was sprayed and allowed to dry at RT.

**Formulation and compaction of HCE tablets**

**Preparations of HCEP**

Adsorption of HCE was tested on two adsorbents: corn starch (Sandhu and Singh, 2007) and SMCC90 (Tobyn et al., 1998), to obtain suitable powder for direct compression. HCE and adsorbents were mixed at the ratio according to Table 1 in a mortar. Each mixture was dried at 50°C for 20 h, then evaluated and selected.

**Table 1. Ratio of HCE and adsorbents**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Ratio of HCE: Adsorbent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corn starch</td>
</tr>
<tr>
<td>1</td>
<td>1:24</td>
</tr>
<tr>
<td>2</td>
<td>1:12</td>
</tr>
<tr>
<td>3</td>
<td>1:8</td>
</tr>
<tr>
<td>4</td>
<td>1:4</td>
</tr>
<tr>
<td>5</td>
<td>1:1</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
</tr>
</tbody>
</table>

**Formulation of HCE tablets**

HCEP (80%) was mixed with a number of pharmaceutical excipients at various combinations (Table 2). Each formulation was subjected to tableting via a hydraulic press. Tablets were then evaluated according to standard methods described in Pharmacopeia.
Table 2. Composition of various HCE tablet formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>HCEP</th>
<th>Avicel pH101®</th>
<th>Encompr®</th>
<th>Purified Talcum</th>
<th>Magnesium Stearate</th>
<th>Ac-Di-Sol®</th>
<th>Explotab®</th>
<th>Aerosil 200®</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>16</td>
<td>1.6</td>
<td>2</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>16</td>
<td>-</td>
<td>2</td>
<td>0.4</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>16</td>
<td>-</td>
<td>2</td>
<td>0.4</td>
<td>-</td>
<td>1.6</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>12</td>
<td>-</td>
<td>4</td>
<td>0.4</td>
<td>-</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>16</td>
<td>-</td>
<td>2</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>1.6</td>
</tr>
<tr>
<td>6</td>
<td>80</td>
<td>13.6</td>
<td>-</td>
<td>2</td>
<td>0.4</td>
<td>1.6</td>
<td>-</td>
<td>2.4</td>
</tr>
<tr>
<td>7</td>
<td>80</td>
<td>16</td>
<td>-</td>
<td>2</td>
<td>0.4</td>
<td>-</td>
<td>1.6</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>80</td>
<td>12</td>
<td>-</td>
<td>4</td>
<td>0.4</td>
<td>-</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>80</td>
<td>12</td>
<td>-</td>
<td>4</td>
<td>0.4</td>
<td>-</td>
<td>1.6</td>
<td>2</td>
</tr>
</tbody>
</table>

Note: SMCC90: HC extract ratio were 3:1 for formulation 1-4, 2:1 for formulation 5-8 and 4:1 for formulation 9.

Quality controls of HCEP, bulk powder and HCE tablets

Quality control of HCEP
The moisture content (%LOD) and flowability of HCEP were determined according to methods described in USP25/NF18.

Quality control of bulk powder
The moisture content, bulk density, tapped density, compressibility ratio and flowability of HC bulk powder were determined.

Quality control of HCE tablets
HCE tablets were subjected to determination of weight variation, hardness, friability and disintegration time according to USP25/NF18. Profile and stability of active compounds in HCE tablet extract were evaluated by chromatographic techniques, using rutin and quercetin as markers.

Stability tests of HCE tablets
HCE tablets were stored at RT and 45°C for 3 months. At the end of each month, tablets stored at 45°C were sampled and the weight variation, hardness, friability, and disintegration time were determined. Tablets stored at RT were analyzed at the end of the 3rd month. Chemical stability was also assessed by thin-layer chromatographic (TLC) analysis.
Statistical analysis
The data are presented as mean±S.D. while Pearson correlation coefficient, means, standard deviations (S.D.) and statistical differences were evaluated through two-way analysis of variance (ANOVA). The SPSS software package (Version 11.0, Chicago, IL) was used for the analysis with \( p \) value <0.05 as statistical significance.

RESULTS AND DISCUSSION

Preparation of HCE
HCE was prepared from aerial parts of HC plants, which were reported to contain high levels of flavonoid-glycosides (Eui et al., 1996; Bansiddhi et al., 2003). HCE appeared as dark-green, highly viscous liquid with unique odor. The yield was 5.85% based on dried HCP.

Calculation of HCE weight in tablets
One 350-mg capsule of GPO NaturePlex® composed 65.3% of five herbal extracts: Borassus flabellifer Linn., Houttuynia cordata Thunb., Randia siamensis Craib., Combretum quadrangulare Kurz. and Mimusops elengi Linn. HC extract accounted for 7.69% of total extract. Since the recommended dose was 2 capsules per day (Sriwanthana et al., 2007), the amount of HC extract per day was 35.16 mg. A no.0 capsule, which holds an average of 278.46 mg HC powder (data not shown), contains an equivalence of 16.38 mg HCE based on a 5.85% yield.

Development of HCE tablets

Preparation of HCEP
Despite high ratio of extract-to-adsorbent, HCEP obtained from formulations 1 and 2 exhibited poor appearance and flow property. At lower ratio in formulations 3-5, wetness was observed and production of granules was difficult. Corn starch is known to be a primary excipient for oral solid dosage form and a common absorbent in pharmaceutical products (Rowe et al., 2003), with adsorption ability resulting from the interaction between free hydroxyl groups (OH) in glucose unit and water molecules via hydrogen bond (Beery and Ladisck, 2001). However, this interaction occurred only on the surface (van den Berg et al., 1975; Beery and Ladisck, 2001) and thus, a high amount of corn starch was required to obtain HCEP with proper characteristics. In contrast, formulations 6 and 7 which utilized SMCC90 as adsorbent showed better results. This is in agreement with a report by Rowe et al., (2003) which suggested the use of SMCC90 as filler for both capsule and tablet forms to improve the compressibility in wet-granulation and direct compression. SMCC90 was obtained from silicification of 2% colloidal silicon dioxide (CSD) and 98% microcrystalline cellulose (MCC). Although its polymorphism, porosity and particle size were not different from those of MCC (Tobyn et al., 1998; Luukkonen et al., 1999), the surface area of SMCC90 was five times higher than that of MCC. SMCC90 was a more effective adsorbent than corn starch because CSD on SMCC90 possessed high affinity sorption sites.
The surface area of CSD in SMCC90 was at 50-380 m²/g (BET method) while that of corn starch was 0.41-0.43 m²/g. In addition, corn starch was insoluble in both cold water and cold 95% ethanol while SMCC90 was soluble in water, organic solvent and acid (Rowe et al., 2003). As a result, less amount of SMCC90 was required to obtain similar HCEP compared to the use of corn starch. This allowed the formulation of smaller size tablet with the same or higher amount of HCE. Formulation 6 was the least ratio of HCE on adsorbent to completely adsorb and showed a good appearance as powder (Table 3), although the flowability remained poor and required further improvement in the formulation process.

Table 3. Appearances of adsorbed HCE formulations (HCEP)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Appearance of HCEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>powder, poor flowability, light green</td>
</tr>
<tr>
<td>2</td>
<td>powder, very poor flowability, green</td>
</tr>
<tr>
<td>3</td>
<td>dried granules, soft, very poor flowability, dark green</td>
</tr>
<tr>
<td>4</td>
<td>dried granules, soft, very poor flowability, dark green</td>
</tr>
<tr>
<td>5</td>
<td>dried granules, hard, passable flowability, dark green</td>
</tr>
<tr>
<td>6</td>
<td>powder, poor flowability, dark green</td>
</tr>
<tr>
<td>7</td>
<td>powder, poor flowability, green</td>
</tr>
</tbody>
</table>

Based on the appearance of HCEP, formulations 4, 6 and 7 were selected for further study (Figure 2). Each formulation was tabulated via hydraulic press with 1- and 2-ton forces. Tablets of formulation 4 appeared too soft as the hardness was immeasurable while tablets of formulation 6 and 7 achieved acceptable hardness. The results also suggested that SMCC90 helped increase the tablet hardness (Table 4).

Figure 2. HCEP formulation 4 (A), 6 (B) and 7 (C)
Table 4. Hardness of HCEP tablets without addition of other excipients (mean±S.D.)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Compression Force (Ton)</th>
<th>Hardness (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>32.80±03.11</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30.10±00.00</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>53.95±08.84</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>48.60±10.64</td>
</tr>
</tbody>
</table>

NA – not applicable, due to tablet softness

**Formulation of HCE tablets**

Due to poor flowability and compaction properties of HCEP, addition of pharmaceutical excipients was required to enhance powder properties suitable for direct compression. These added excipients were chemically inert, causing neither interaction with nor decomposition to the active ingredients in the extract (Jivraj et al., 2000). In the formulation, microcrystalline cellulose (Avicel PH101®) was used as binder/disintegrant. A study by Palma et al., (2002) on a formulation of tablet from plant extract showed that Avicel PH101® facilitated the disintegration of tablets. Dibasic calcium phosphate (Emcompress®), with a good flowability and compact property was utilized as glidant (Rowe et al., 2003). Magnesium stearate served as lubricant and antiadherent (Eilalifa et al., 2009). Sodium carboxymethyl starch (Explotab®) or crosslinked sodium carboxymethylcellulose (Ac-Di-Sol®) was employed as superdisintegrant. Purified talcum possessed lubricating and antiadherent properties. Colloidal silicon dioxide (Aerosil 200®) was used as glidant and antiadherent to improve flowability and content uniformity of pre-compressed powder (Gierer, 2002; Rowe et al., 2003; Teng et al., 2009). A decrease of Avicel PH101® and increases of purified talcum and addition of Aerosil 200® in formulation 4, resulted in tablets with higher hardness and longer disintegration time (>15 min) than other formulations. Tablets of formulations 8 and 9 appeared to have acceptable hardness, disintegration time (<15 min) and friability (<1%) (USP25/NF18). The properties of formulated tablets are compiled in Table 5.
Table 5. Hardness, disintegration time and friability percentage of HCE tablets

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Hardness (N)</th>
<th>Disintegration time (min)</th>
<th>% Friability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47.28±5.39</td>
<td>2.55</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>31.72±2.56</td>
<td>0.35</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>54.37±2.75</td>
<td>1.52</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>97.06±8.53</td>
<td>&gt;15</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>21.61±2.62</td>
<td>1.02</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>23.76±3.36</td>
<td>0.45</td>
<td>0.06</td>
</tr>
<tr>
<td>7</td>
<td>14.44±1.71</td>
<td>0.18</td>
<td>0.22</td>
</tr>
<tr>
<td>8</td>
<td>22.15±1.70</td>
<td>6.32</td>
<td>0.08</td>
</tr>
<tr>
<td>9</td>
<td>57.98±7.60</td>
<td>2.18</td>
<td>-0.04</td>
</tr>
</tbody>
</table>

ND – not determined

Quality controls of HCEP, bulk powder and HCE tablets

Quality controls of HCEP and bulk powder

The moisture content of bulk powder was 37.6% lower that of HCEP (Table 6). High moisture content of herbal tablets was reported to associate with the growth of microorganisms and possibility of degradation of active compounds (Sitthichai, 2004). Lower moisture content also contributed to the improvement of the flow property as evidenced by a significant decrease in the angle of repose. The compressibility ratio of bulk powder was within a range (5-12%) that suggested excellent flowability. The properties of this bulk powder appeared to be suitable for direct compression (Jivraj et al., 2000).

Table 6. Quality controls of HCEP and bulk powder

<table>
<thead>
<tr>
<th>Test</th>
<th>HCEP</th>
<th>Bulk powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD (%)</td>
<td>5.29</td>
<td>3.30</td>
</tr>
<tr>
<td>Bulk density</td>
<td>-</td>
<td>0.398</td>
</tr>
<tr>
<td>Tapped density</td>
<td>-</td>
<td>0.431</td>
</tr>
<tr>
<td>Compressibility ratio (%)</td>
<td>-</td>
<td>7.609</td>
</tr>
<tr>
<td>Repose angle (˚)</td>
<td>46.20±4.80</td>
<td>28.10±2.60</td>
</tr>
</tbody>
</table>

Quality controls of HCE tablets

Weight variation of HCE tablets was within the range (USP25/NF18), thus ensured the consistency of the amount of active compounds of HCE tablets. The hardness of tablets was invariable. The friability and disintegration time were less than 1% and 15 min, respectively (Table 7), both were also within acceptable range (USP25/NF18). These parameters are important in commercialized process as they directly influenced the quality and shelf-life of the products (Sitthichai, 2004).
Table 7. Quality controls of HCE tablets

<table>
<thead>
<tr>
<th>Weight (mg)</th>
<th>Diameter (mm)</th>
<th>Thickness (mm)</th>
<th>Hardness (N)</th>
<th>Friability (%)</th>
<th>Disintegration time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250.26±0.73</td>
<td>8.51±0.01</td>
<td>3.40±0.01</td>
<td>41.50±3.20</td>
<td>0.04</td>
<td>1.23</td>
</tr>
</tbody>
</table>

Data are expressed as mean±S.D.

Stability tests of HCE tablets

**Stability test of HCEP and bulk powder**

Under both 45˚C and RT storage conditions, storage time affected the moisture content of HCEP and bulk powder. The longer the storage time, the lower the moisture content (Table 8). Compared to the value on 0 month (Table 6), % LOD of HCEP kept for 3 months at 45˚C and RT were decreased 58.6 and 59.7%, respectively. The trend also applied to the bulk powders, in which the decreases were 33.6 and 37.6% for the 3-month storage at 45˚C and RT, respectively. The appearances of the HCEP and bulk powder remained unchanged. In the 1st month period, the rate of moisture evaporation was the highest especially at 45˚C. This was because HCEP and bulk powder were exposed to a relatively high temperature, thus a rapid transfer of moisture within the system occurred to balance the temperature. As the temperature of the samples were in equilibrium with the storage system, the rate of moisture evaporation decreased (2nd and 3rd month) (Sirithunyalug et al., 2008).

Table 8. Moisture content (%LOD) of HCEP and bulk powder

<table>
<thead>
<tr>
<th>Conditions</th>
<th>HCEP</th>
<th>Bulk powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>45˚C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Mo</td>
<td>2.71</td>
<td>2.64</td>
</tr>
<tr>
<td>2 Mo</td>
<td>2.43</td>
<td>2.50</td>
</tr>
<tr>
<td>3 Mo</td>
<td>2.19</td>
<td>2.19</td>
</tr>
<tr>
<td>RT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Mo</td>
<td>2.13</td>
<td>2.06</td>
</tr>
</tbody>
</table>

**Stability of HCE tablets**

Quality controls of HCE tablets after stability test are presented in Table 9. Compared to the data of 0-month tablets (Table 7), the weights of tablets were not significantly different as the storage time was extended. Storage conditions showed no effect on the weight and friability of tablets. In contrast, the hardness of tablets stored at 45˚C was affected by the storage time. The hardness of tablets was significantly decreased after one month of storage and continued to slowly but significantly decrease as the storage time was extended. This was in concurrent with a slight decrease in disintegration time of tablets after 2 months which suggested a possibility of tablets taking up moisture, resulting in a more water-penetrable structure and a more rapid disintegration rate. A significant increase in disintegration time of tablets stored at RT for 3 months was also a result of an increase in the hardness of tablets.
Table 9. Quality controls of HCE tablets after stability test

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Weight (mg)</th>
<th>Dimension (mm)</th>
<th>Thickness (mm)</th>
<th>Hardness (N)</th>
<th>Friability (%)</th>
<th>Disintegration time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Mo</td>
<td>246.96±1.17</td>
<td>8.50±0.00</td>
<td>3.43±0.02</td>
<td>37.50±2.10</td>
<td>0.01</td>
<td>1.20</td>
</tr>
<tr>
<td>45˚C 2 Mo</td>
<td>246.13±1.48</td>
<td>8.52±0.01</td>
<td>3.43±0.01</td>
<td>36.50±1.00</td>
<td>-0.01</td>
<td>1.25</td>
</tr>
<tr>
<td>3 Mo</td>
<td>245.76±1.10</td>
<td>8.51±0.01</td>
<td>3.43±0.02</td>
<td>34.70±1.00</td>
<td>-0.20</td>
<td>1.02</td>
</tr>
<tr>
<td>RT 3 Mo</td>
<td>246.84±1.22</td>
<td>8.49±0.02</td>
<td>3.37±0.01</td>
<td>44.90±4.20</td>
<td>-0.07</td>
<td>2.18*</td>
</tr>
</tbody>
</table>

Data are expressed as mean±S.D., (*p<0.05)

Stability of chemical components in HCE tablets was tested by TLC analysis using rutin and quercetin as standard markers. Tablet extracts showed TLC profiles that were practically identical to that of the crude extract, at both RT and 45˚C during the 3-month storage, suggesting that no degradation or decomposition of the extract components occurred (Figure 3). This is in part due to the elimination of solvent from the extract to prevent chemical reaction and the minimization of moisture content in the adsorption step which prevented the growth of microorganisms that could lead to fermentation and chemical decomposition (Sitthichai, 2004). The results also verified the compatibility between the chemical components in the extract and the excipients used in the formulation of tablets.

Figure 3. TLC chromatograms of HCE tablet extracts, compared with crude extract and standards. Chromatograms are visualized under UV-254 nm (A) and UV-365 nm (B). Lane 1: crude HCE, Lane 2: HCE tablet extract 0th Mo at 45˚C, Lane 3: HCE tablet extract 1st Mo at 45˚C, Lane 4: HCE tablet extract 2nd Mo at 45˚C, Lane 5: HCE tablet extract 3rd Mo at 45˚C, Lane 6: HCE tablet extract 3rd Mo at RT, Lane 7: rutin standard, Lane 8: quercetin standard.
CONCLUSIONS

Formulation of physically- and chemically-stable *H. cordata* extract (HCE) tablets was accomplished. Preparation of the plant extract eliminated microbial contamination from the raw materials and the active components can be concentrated to as high as 4 folds compared to that of a HCP capsule. Standardization of the extract with chromatographic methods using flavonoid standards as markers allowed the preparation of uniformed, consistent and reproducible HCE tablets. Selection of an appropriate adsorbent, in this case SMCC90, aided the conversion of viscous, sticky plant extract into low moisture, compressible powder. With the addition of other suitable pharmaceutical excipients, powder with good flowability and compressibility was obtained for manufacturing of HCE tablets with good appearances and disintegration. The prepared tablets showed good stability at both RT and at 45°C conditions over the period of 3 months. HCE tablets can be used as a food supplement to replace the traditional powder capsules. The process and techniques from this study can also be employed in the development of other medicinal plant extract tablets.

ACKNOWLEDGEMENTS

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Versatile Compression Force Measuring System for Rotary Tablet Presses

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ABSTRACT

Compression force has become more important in in-process quality control of tablet compression. In this study, two different designs of industrial rotary tablet presses were tested for versatility of the designed force measurement systems. Both presses were equipped with force measuring systems designed by using commercially-available equipment and instruments so that the procedure can be easily reproduced for commercial applications. A remote installation technique was used for installing an HBM® load cell at the compression roller’s eyebolt. A bridge amplifier, analog to digital converter, and microcontroller from TSM® were used to determine the average maximum compression force, with the result displayed on a 4- digit LED display. C programming language was used to develop the program for the microcontroller. The calibration method was designed by installing a calibrated HBM® load cell on a modified lower punch, then manually pressing a modified upper punch onto the load cell with static force and collecting data for making the calibration curve. A linear relation between the punch’s load cell and the data collected on the microcontroller resulted from both machines’ calibrations. Testing the calibrated machine by applying static force comparable to the LED display was done to study the reliability of the calibrated force measuring system. Two direct compressible diluents, lactose and microcrystalline cellulose, were compressed into 12 mm, flat, bevel-edged tablets at the normal production speed of 400 tablets/minute at various compression forces on both calibrated tablet presses. Both the pressure-tensile strength profile and the pressure-tablet thickness profile were studied. The results were similar to work of other researchers while the tablet compression process was done according to industrial standards. This proved that the retrofitted machines could be used for both research work and industrial tablet compression.

Key words: Compression Force, Rotary Tablet Press, Versatile
INTRODUCTION

Rotary tablet presses are essential machines for pharmaceutical tablet production. They all provide basic functions and controls to make good tablets but the majority of them do not have a compression force measuring system. Compression force is an important parameter in determining the quality of tablets in a quality assurance process. The demand for such a system has been increasing but installation requires engineers with special skills in mechanical, electrical, and computer technology working together. Given this, the availability of this technology is limited and the price very high. With the Thai pharmaceutical industry currently employing many rotary tablet presses, the purpose of this study is to design a simple, versatile, compression force measuring system that can be retrofitted into these presses with proven versatility and reliability. This will help the Thai pharmaceutical industry elevate their quality assurance standards.

Higuchi et al., (1954) instrumented an eccentric tablet press and began the development of compression force measurement in 1954. In the early stages of development, most compression force measurements were done on eccentric presses because the instrumentation could be placed directly on the punch. Levin (2002) discussed the uses of instrumentation for formulation development but when products developed on eccentric presses were scaled-up in rotary presses, the results were different due to differences in compression and the feeding mechanism. In a rotary press, the punches are moving with the turret, making it more difficult to instrument. Watt (1988) described different ways of instrumentation of a rotary press. Shotton et al., (1963) instrumented upper and lower punches, then transmitted the signals via radio and recorded them on an ultraviolet recorder. Knoechel et al., (1967) instrumented a rotary press at a remote site and recorded it on an oscilloscope. Goodhart et al., (1968) compared various sites of remote installation in rotary presses. Patel et al., (2007) used infrared telemetry to transmit information from both upper and lower punches rotating on a turret. A summary of instrumentation of rotary tablet press is shown in Figure 1.

Researchers used different instrumentation techniques to measure compression force but all of them used a thin metal foil strain gauge to detect force by cementing it to the punches or remote parts through which the forces were transferred. This method was difficult to reproduce and maintain in an industrial application. Perry and Lissner (1962) stated the need to protect strain gauges from moisture and hazardous conditions that would change the characteristics of strain gauges. Watt (1988) discussed the advantage of using an industrial load cell that was well protected in a sealed case for easy maintenance and that could be easily changed if damaged. Industrial load cells became popular. They are widely used in electronic balances but rarely used for instrumentation of tablet presses because parts on the presses need to be modified to fit with the load cells.
Figure 1. Instrumentation of a rotary tablet press
In 1984, Van Aerde (1984) published his work about instrumentation of compression force using microprocessor-based data acquisition and successfully used a computer to handle data for compression force measurement. This started a new era of using a microcontroller and computer in compression force measurement. Figure 2 shows different ways to display compression force.

**Figure 2.** Different ways to display compression force
In this experiment, remote installation technique was used to instrument eyebolts of lower compression rollers with industrial load cells in two rotary tablet presses. A commercially-available bridge amplifier, analog to digital converter (A/D converter), microcontroller, and digital display were used to determine and display the compression force. The principle of the work is shown in Figure 3.

**Figure 3.** Principle to measure compression force in rotary tablet presses

**EXPERIMENTAL DESIGN**

Two tablet presses, Manesty B3B (Manesty Machines Limited, Liverpool, England) and Narong NRIR13D (N.R. Industries Co., Ltd., Thailand), were selected for this study. Eyebolts of both machines were studied and redesigned to accommodate a force transducer with strain gauge (load cell) HBM® Model U9B 20 kN (Hottinger Balbwin Messtechnik GmbH, Germany). After installing the load cells, both of the eyebolts still had the same function as before, only that the load cells were acting as a part of them. The load cells could measure any force transferred from the compression rollers through these eyebolts. Bridge amplifiers and Analog to Digital converters (A/D converter) (ELZET TSM-LOADCELL®, ELZET80 Mikrocomputer GmbH & Co., KG, Germany), were used to amplify the load cells’ signal and convert the force analog signal into digital data. Force
digital data were then interpreted and calculated by microcontroller (ELZET TSM-CPU® model 32 H2EA, ELZET80 Mikrocomputer GmbH & Co., KG, Germany). The average maximum force was displayed on a four-digit, serial LED display model SC4Dlite A (Silicon Craft Sdn. Bhd., Malaysia). The installation of this equipment is shown in Figure 3.

Compression of lubricated lactose SUPER TAB SD® (DMV – Fonterra Excipients GmbH & Co., KG, Germany), was performed on NRIR13D at a slow speed of 160 tablets/minute. An oscilloscope (Tektronix model TDS2012, Tektronix Inc., Oregon, USA) was used to study the waveform of the compression force and a photograph of the frozen waveform was taken. A C-language program was developed according to the result of the study of the waveform. The developed program was then downloaded to the TSM-CPU®.

The calibration method was designed by installing a calibrated 20 kN HBM® load cell on a modified lower punch, then manually pressing a modified upper punch onto the load cell to create static force. The modifications of the upper and lower punch are shown in Figure 4. The static force created at the punches was then detected at the load cell installed at the eyebolt. A measuring amplifier (model SCOUT55®, Hottinger Balbwin Messtechnik GmbH, Germany) was used to read the static force on the calibrated load cell while the TSM-CPU® sent the digital data received from the load cell on the eyebolt. The principle of this calibration procedure is shown in Figure 5. The amplification factor of each machine was set up. An amplification factor of 20 kN to ¼-full scale of 0-4095 (12 bit) was set for the B3B press and an amplification factor of 20 kN to ½-full scale of 0-4095 (12 bit) was set for the NRIR13D press. The settings would allow the B3B press to measure up to a maximum force of about 80 kN (8 tons) and the NRIR13D press up to 40 kN (4 tons). This technique was prepared for adapting the compression force measuring system to fit with presses of various maximum compression forces.

Static forces of 1, 2, 3, 4, 5, 10, 15, and 20 kN were applied to the standard load cell. Readings from the SCOUT55® and the corresponding digital data received from the CPU were recorded. The force values were changed from kN to hN for plotting calibration curves of both machines. The slope and intersection point of each curve were used for calculating compression force in the C language programs written for the corresponding machine.

Two preparations, each of 5 kg, of direct compressible diluent, microcrystalline cellulose, MCC, (Comprecel® Mingtai Chemical Co., Ltd., Taiwan ROC) were prepared by adding and mixing 1% of magnesium stearate (UNION DERIVAN, SA (UNDESA), Spain). A dry blender (double cone dry blender model DB-15, N.R. Industries Co., Ltd., Thailand) was used for mixing the preparation for 5 min each. The same steps were carried out for lactose (SuperTab SD®, DMV – Fonterra Excipients GmbH & Co., KG, Germany).

Both calibrated machines, B3B and NRIR13D, were used to compress 560 mg. tablets of lubricated MCC and lactose with 12.0 mm flat bevel-edged punches and dies. The punches and dies for B3B were B type while for the NRIR13D were D type. Average compression forces of 4, 6, 8, 10, and 15 kN were applied
Figure 4. Modification of upper and lower punches for calibration

Figure 5. Principle of compression force calibration
for MCC tablets and average compression forces of 6, 8, 10, 15, 20, and 25 kN were applied for lactose tablets. Twenty sample tablets were collected at each compression force. A total of 200 tablets were collected for MCC and 240 tablets for lactose. Each tablet was weighed on an electronic balance (SARTORIUS® Model ED 323S, Satorius AG, Switzerland). The thickness of each tablet was measured with a digital vernier caliper (model BD-10, Baker Gauges India Pvt. Ltd., India). The hardness of the tablets was measured using a hardness tester (model 5Y, Dr. Schleuniger Pharmatron AG, Switzerland). Data from all the measurements were collected. Studies of tablets’ weight variation, changes of thickness due to changes of compression pressure, and changes of tablets tensile strength due to changes of compression pressure were carried out.

RESULTS AND DISCUSSION

The two tablet presses, as shown in Figure 6, were selected to represent the tablet presses used in the Thai pharmaceutical industry. Their designs are different. The Manesty B3B machine has a unique lower compression roller eyebolt built outside of the machine’s main frame while the Narong NRIR13D has its lower compression roller eyebolt built inside the machine’s frame. The B3B represents tablet presses from Manesty, England; Stroke, USA; Cadmach, India, and some manufacturers from China. The NRIR13D represents the presses Kilian, Korsch, and Fette, Germany; Jen Chiang, Taiwan; and many manufacturers from China. Both of them use a spring for overload protection but with different mechanisms of tablet thickness adjustment. The differences of the two machines potentiate the versatility of the force measurement system designed in this study. The other important difference of the two is the tableting tool (American Pharmaceutical Association, 1995). The B3B uses B type tooling while the NRIR13D uses D type tooling. The head flat, the flat part of a punch head, of D type tooling is 15 mm. in diameter while the B type is 10.0 mm. During the compression cycle, D type tooling will have a 50% longer dwell time than the B type. This enhanced the value of this study because the effect of dwell time on a tablet’s properties could also be studied. One other difference of design is that the two machines use different granule feeding systems. The B3B uses a gravity feed shoe while NRIR13D uses a forced feeder. A weight variation study was carried out to prove that the differences in the feeding systems did not effect tablet weight variation.
Many researchers have used metal-foil resistance strain gauges for measuring tablet compression force. Its advantage is that it can be cemented to the part nearest to the force origin for precise force measurement. However, the procedure to cement the metal-foil to the part needs competent, skillful, and very fine workmanship. Reproducibility of the work is not very likely because it depends very much on skill. In this experiment, a remote installation method was selected for installation of the force transducer. An industrial load cell was selected for force transducer instead of a metal-foil resistance strain gauge because it has some advantages over metal-foil. Watt (1988) described the advantages of load cells for industrial uses in that the device was sealed for protection from dust and moisture and can be replaced easily when damaged. Its other advantage is that the elasticity of the load cell is tested from the factory and the linearity of it must be confirmed. The disadvantage of the load cell is its large size. It needs more space than a metal-foil strain gauge. Due to its large size, installation directly at the punch is not possible. In this experiment, eyebolts of both machines were redesigned so that they were separated into two pieces to accommodate the load cells, HBM® Model U9B 20 kN, in the middle. The screws on each side of the load cells were tightened to each piece of the eyebolt to make the completed eyebolts similar to the original eyebolt in terms of length and their application. The only difference was that the new ones, shown in Figure 7, have the load cells installed in the middle to detect forces transferred through them.

Figure 6. Tablet presses – Manesty B3B (left) and Narong NRIR13D (right)
Figure 7. The load cell installed on the Manesty B3B (left) and Narong NRIR13D (right)

The compression force waveform of NRIR13D is shown in Figure 8. The compression speed was 160 tablets/minute. The cycle time of one compression cycle was about 380 ms of which the compression duty time was about 270 ms and the remaining idle time was about 110 ms. Van Aerde et al., (1984) used a 12-bit A/D converter with the sampling time adjustable from 600 µs to 16 s. The average sampling rate of the microprocessor was 500 samples per channel per compression cycle and the data collected gave very fine waveform plotting for compression force study. In this experiment, the A/D converter (ELZET80® Mikrocomputer GmbH &Co., Germany using microchip MAX197®, Maxim Integrated Products, USA) could convert analog signal into 12 bit (0-4095) digital data with the conversion time of 6 µs. The microcontroller used in this experiment was an ELZET TSM-CPU 32 H2EA (ELZET80 Mikrocomputer GmbH & Co., Germany), with a sampling rate every 100 µs. When the tablet press was running at 400 tablets/minute, the total number of samples per cycle was about 1,500 samples. From the study of the waveform, it could be concluded the performance of the data collecting system exceeded that needed to properly measure compression force for either industrial or research applications.
Computer programs were developed using C programming language to monitor the presses and compression force. Modular programming (Hanly and Koffman, 2001) was used so that the high-speed tasks and the administrative tasks were separated for better performance. The high-speed task consisted primarily of the maximum forces detection. This part starts from receiving a trigger pulse from the mCAT (Mocom Software GmbH & CO KG., 2006), the operating system used in the ELZET TSM-CPU 32 H2EA, every 100 µs. Then it gets data from the A/D converter and analyzes the data to find the maximum point. In order to reduce the CPU occupied time, the compression wave was divided into 3 zones as shown in Figure 9. In rising zone 1, the data received from the A/D converter kept on increasing 50 times, confirming that the compression force was on the rising edge and started zone 2. The program started comparing new data with previous data, the higher value was stored in the maximum value variable until the highest value was reached. The zone 3 started when the program would count the number of data that decreased from the maximum points for 50 times, then sending the maximum value to the main program and stopped to wait for zone 1 of next wave. In the event that zone 3 did not occur within 3 sec (during calibration with the static force), the program would send out the current existing value.

**Figure 8.** Compression force waveform of NRIR13D
When the presses started, the computer program managing the administrative task would look for the proximity signal from punch number one on the press to come in and would synchronize the data table with the punch number of the press. This occurred with every turn of the turret to ensure the punch number and data were always synchronized. This routine began when the maximum value was sent from the high-speed task. The calculation of absolute compression force was done using the slope and intersection from the calibration curve discussed in the next paragraph. Programs for both machines were the same except for the constants used in the force calculation equation. The programs were written using event-driven programming (Ferg, 2006) techniques so that the program would stay idle unless the event it was waiting for occurred. For the high-speed task, the event was the trigger signal from mCAT. For the administrative tasks/program, the events were the proximity signal from punch number one (only one time per round) and the maximum value from the high-speed task, which occurred once each compression cycle. This technique reduced the processing time of the CPU to keep its performance at maximum. The average of maximum force was displayed in kN with an accuracy of 0.1 kN.

To reduce the data processing and transfer time between the CPU and the LED display, another technique was developed. If the value was sent in kN, then it had to be transferred in floating point which took much longer than data transferred in integer. In this experiment, the average of maximum force was sent in hN (equal to 0.1 kN) to a 4-digit LED display via RS232. The display was configured to display a decimal point in between the third and the last digit. This way, the result was automatically converted back in kN with the accuracy of ± 0.1, while the data processing and transfer were done in integer to save the processing time.
Since the compression force was measured from a remote site, a calibration procedure had to be developed to compare the force at the punches and the value received at the CPU. The procedure for calibrating the force measuring system was similar to that developed by Sirithunyalug et al., (1999). The results of the calibration are shown in calibration curve in Figures 10 and 11. The slope and intersection for B3B was determined as: \( Y = 0.2076 \times X - 16.2783 \) (Coefficient of determination \( R^2 = 0.999 \)). The slope and intersection for NRIR13D was determined as: \( Y = 0.0985 \times X - 7.9442 \) (Coefficient of determination \( R^2 = 1.000 \)).

**Figure 10.** Calibration slope and intersection of the Manesty B3B press

**Figure 11.** Calibration slope and intersection of the Narong NRIR13D press
The linearity of both curves was very good due to the linear elasticity of the load cell. The intersections of the curves were not at the 0,0 point because the weight of the compression rollers and accessories were always pressing on the measuring load cells at zero punch force. The slope and intersection obtained from the calibration curves were used by the CPU to calculate compression force. Even though the amplification factors of both presses were different, the calibrations of both presses were linear. The setting of 20 kN to ¼ full scale of 0 - 4095 (12 bit) was for B3B while the NRIR13D was set to ½ full scale of 0 - 4095 (12 bit) at 20 kN. The settings allowed the B3B press to measure up to a maximum force of about 80 kN (8 Tons) and the NRIR13D press up to 40 kN (4Tons). This technique allowed for adapting the compression force measuring system to fit presses of various maximum compression forces.

After the calibration processes were completed, the machines were tested again with the same standard load cell and amplifier to compare the reading with the compression force read at the LED display of the systems. The results of these comparisons are shown in Tables 1 and 2. The average error of the reading is 0.47% for both machines with a maximum error of 1.06% for the B3B press and 1.01% for the NRIR13D press.

Table 1. Comparing the standard load cell with LED display value of Manesty B3B

<table>
<thead>
<tr>
<th>Scout reading (Master) [kN]</th>
<th>Force Display (on LED) [kN]</th>
<th>Error [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0017</td>
<td>0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>5.1396</td>
<td>5.1</td>
<td>0.77</td>
</tr>
<tr>
<td>10.2734</td>
<td>10.3</td>
<td>-0.26</td>
</tr>
<tr>
<td>15.3217</td>
<td>15.2</td>
<td>0.79</td>
</tr>
<tr>
<td>20.2145</td>
<td>20.0</td>
<td>1.06</td>
</tr>
<tr>
<td>Average error</td>
<td></td>
<td>0.47</td>
</tr>
</tbody>
</table>

Table 2. Comparing the standard load cell with LED display value of Narong NRIR13D

<table>
<thead>
<tr>
<th>Scout reading (Master) [kN]</th>
<th>Force Display (on LED) [kN]</th>
<th>Error [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0012</td>
<td>2</td>
<td>0.00</td>
</tr>
<tr>
<td>5.0435</td>
<td>5.0</td>
<td>0.86</td>
</tr>
<tr>
<td>10.1644</td>
<td>10.2</td>
<td>-0.35</td>
</tr>
<tr>
<td>15.4278</td>
<td>15.3</td>
<td>0.83</td>
</tr>
<tr>
<td>20.1034</td>
<td>19.9</td>
<td>1.01</td>
</tr>
<tr>
<td>Average error</td>
<td></td>
<td>0.47</td>
</tr>
</tbody>
</table>
Most researchers send their instrumented parts to be calibrated by a calibrating machine before installing on the tablet presses. The calibration technique developed in this experiment was different in that the instrumented parts could be calibrated when on the press. This is very important because Good Manufacturing Practice (GMP) regulations require all equipment to be validated before releasing for use in production or R&D. Calibration, which is a part of the validation process, must be performed annually to ensure that parameters affecting the quality of products are well controlled. The calibration process designed in this experiment makes it possible to fulfill the GMP regulation requirements.

Two commonly used, direct-compressible diluents, MCC and lactose, were used to make tablets at various compression forces. The forces were then converted into pressure, based on the punch diameter of 12.00 mm. Hardness of tablets also was converted into tensile strength, using the equation proposed by Haririan and Newton (1999) for flat round tablets:

\[ T = \frac{2P}{\pi D t}, \]

where \( T \) = Tensile strength, \( P \) = Fracture load, \( D \) = Diameter, and \( t \) = Thickness

In this experiment, after setting the weight and compression force, tablets were collected. The thickness of tablets was kept constant throughout each compression pressure. When thickness is kept constant, weight variation of the tablets plays a major role in variation of compression pressure, resulting in uncertainty of other tablet properties. Because the two tablet presses had different granule feeding systems – the B3B press used a gravity feed shoe and the NRIR13D press used a forced feeder – the variation in tablet weights was measured to check that both machines were within normal limit. A summary of the weight variation study is shown in Table 3. British Pharmacopeia (2004) defined the acceptable limit of weight variation in uncoated and film-coated tablets as:

- Tablets 80 mg or less: Percentage Deviation 10%
- Tablets more than 80 and less than 250 mg: Percentage Deviation 7.5%
- Tablets more than 250 mg: Percentage Deviation 5%

### Table 3. Summary of tablet weight variation

<table>
<thead>
<tr>
<th>Standard deviation, average weight, relative standard deviation</th>
<th>Manesty B3B</th>
<th>Narong NRIR13D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>A.W.</td>
</tr>
<tr>
<td>4 kN MCC MCC</td>
<td>2.51</td>
<td><strong>549.10</strong></td>
</tr>
<tr>
<td>6 kN MCC MCC</td>
<td>2.98</td>
<td>564.70</td>
</tr>
<tr>
<td>8 kN MCC MCC</td>
<td><strong>1.93</strong></td>
<td>559.15</td>
</tr>
<tr>
<td>10 kN MCC MCC</td>
<td>2.32</td>
<td>560.30</td>
</tr>
<tr>
<td>15 kN MCC MCC</td>
<td>1.97</td>
<td>560.90</td>
</tr>
<tr>
<td>6 kN Lactose</td>
<td>2.31</td>
<td>562.20</td>
</tr>
<tr>
<td>8 kN Lactose</td>
<td>3.71</td>
<td>561.70</td>
</tr>
<tr>
<td>10 kN Lactose</td>
<td>3.57</td>
<td>56.10</td>
</tr>
<tr>
<td>15 kN Lactose</td>
<td>6.44</td>
<td>559.80</td>
</tr>
<tr>
<td>20 kN Lactose</td>
<td>4.32</td>
<td><strong>563.55</strong></td>
</tr>
<tr>
<td>25 kN Lactose</td>
<td><strong>7.05</strong></td>
<td>552.25</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>3.56</strong></td>
<td><strong>559.43</strong></td>
</tr>
</tbody>
</table>
In this experiment, average tablet weight of all the tablets compressed in the Manesty B3B press was 559.43 mg and 565.59 in the Narong NRIR13D press. The control value was 560 mg. The highest standard deviation in the B3B machine was 7.05, which was equivalent to a relative standard deviation of 1.28%. The highest standard deviation in the NRIR13D machine was 6.01, which was equivalent to a relative standard deviation of 1.06%. The highest tablet average weight was 568.1 mg (+1.45%) in 6 kN MCC tablets made in the NRIR13D press and the lowest was 549.1 mg (-1.95%) in 4 kN MCC tablets made in the B3B machine. The weight variation was well controlled in this experiment.

The pressure-thickness profile of the tablets produced was studied and the results are shown in Figures 12 and 13. Changes in thickness due to the pressure of the two diluents were clearly different. MCC tablets decreased more in thickness with increasing compression pressure while lactose showed a much lower relative response. The results were similar to that of Palanuphap (1995) and Tye et al., (2005). The differences in the deformation mechanisms could explain this. Lactose undergoes fragmentation under compression while MCC undergoes plastic deformation. Under compression force, lactose fragmented before it could consolidate to form a tablet, the void space was reduced significantly before it could convert compression energy into compaction bonding to form a solid tablet. At the pressure at which it could form a solid tablet, the void space was already greatly reduced. MCC, on the other hand, formed hard tablets at lower pressure because plastic deformation and bonding at points of contact occurred together with materials of plastic deformation under compression. At the pressure that MCC formed a tablet, the deformation was still far from completion, resulting in much higher void space than lactose at low compression force. In this experiment, MCC were compressed only up to 15 kN of compression force because the hardness of the tablets was already higher than 400 N at 15 kN. The maximum hardness that the hardness tester could test was 450 N, so for MCC, the maximum force was set at 15 kN.

**Figure 12.** Pressure-thickness profile of MCC tablets
The effect of dwell time on tablet thickness was observed in this experiment. The NRIR13D press used D type tooling which had a higher dwell time than the B type tooling used in the B3B press. The effect of dwell time on changes of thickness was shown on both MCC and lactose; the higher the dwell time, the lower the thickness of tablets. The effect was more pronounced in MCC than in lactose. More interesting was that at high compression pressure of about 220 MPa, the thickness of lactose tablets was the same on both machines because the tablets had reached true density (void space became zero).

Tye et al., (2005) expressed tabletability in terms of a pressure-tensile strength profile. It indicates the capability of a powder to be transformed into a tablet of specified strength under the effect of compaction pressure. In this experiment, a pressure-tensile strength study was conducted on the sample tablets with the results shown in Figure 14. Tabletability of MCC was much higher than that of lactose, which was similar to results found by Palanuphap (1995) and Tye et al., (2005). MCC is well known for its superiority to other diluents in terms of tabletability and is the first choice for use to increase tablet hardness. The effect of dwell time on tabletability was also observed. Narang et al., (2010) did an experiment on lactose and MCC mixture and found that dwell time had effect on tabletability. Rees and Rue (1978) found that dwell time had more effect on lactose than MCC. Tye et al., (2005) stated that MCC was independent of compression speed (dwell time) and there was a minor effect on lactose. The results from this study showed similar results with dwell time having no effect on MCC tabletability but a more pronounced effect on lactose, especially at high compression pressure.

Figure 13. Pressure-thickness profile of lactose tablets
CONCLUSION

Two different types of rotary tablet presses were instrumented with compression force measuring systems that had been specifically designed to be fitted to them. Instrumentation of the presses was done at a remote site. Industrial load cells were used for detecting compression force to avoid complication in the instrumentation process and also for easy maintenance in industrial use. The compression force waveform was studied using an oscilloscope to determine the performance of the force measuring system needed to detect the wave. Commercially available bridge amplifiers, A/D converters, and microcontrollers were used in this study. The computer program was developed in C language to match the mCAT operating system used on the microcontroller. The cycle time of each data collection point from the system was 100 µs, resulting in fine measurement of the waveform. The average of the maximum compression force was calculated and displayed on a 4-digit LED display. Different amplification factors were set for the two presses; the B3B press was set at ¼ full scale and the NRIR13D press was set at ½ full scale for a compression force of 20kN. The calibration tools and method were designed to perform calibration directly at the press. The result of calibration on both presses showed a linear relationship between the compression force on the punch and the value read at the CPU. System reliability was checked by comparing the force shown on the standard load cell and the value of the LED display, which differed at an average of 0.47%. After calibrating, lactose and MCC tablets were compressed at a rate of 400 tablets/minute on both machines. Twenty tablets from each compression force were collected. Studies on weight variation, the pressure-thickness profile, and the pressure-tensile strength of the tablets were carried out. The results were similar to what others had found in prior research. These results proved that the instrumented machines could be used for both research and commercial production of tablets. In addition, the system was versatile, as it could be adapted to different types of presses for use at different amplification factors and for different types of applications. The designed system could be easily reproduced for commercial application with a calibration method that meets GMP regulations.

Figure 14. Pressure-tensile strength profile of the tablets
ACKNOWLEDGEMENTS

The authors would like to thank N.R. Industries Co., Ltd. and its staff for their kind support in this research.

REFERENCES


Dioxouranium(VI) Complexes of Some Monovalent Bidentate Schiff Base Ligands Derived from Aniline

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ABSTRACT

Several new dioxouranium(VI) complexes of Schiff bases [LH], derived from o-hydroxyaldehyde or ketone, 2-hydroxy-1-naphthaldehyde and aniline have been prepared and characterized on the basis of elemental analyses, IR and electronic absorption spectra and magnetic susceptibility measurements. The results suggest that the Schiff base is a monovalent anion with bidentate ON donor atoms of the phenolic oxygen and the azomethine nitrogen atoms. The formulae were found to be UO₂L₂ for the 1:2 non-electrolytic complexes and six-coordinate structure has been proposed for the complexes.

Key words: Aniline, dioxouranium(VI), o-hydroxyaldehyde or ketone, Schiff base

INTRODUCTION

Transition elements of Group IV, V and VI are known to form mononuclear oxocations of the type MOₙ⁺ and MO₂ₙ⁺. The most thoroughly investigated, best characterized and most stable oxometal cations are the dioxouranium(VI), dioxomolybdenum(VI) and oxovanadium(IV) ions. The strongly-bound oxygens of these oxometal cations remain intact during chemical reactions and produce one or more additional absorption bands beyond those normally available in transition metal complexes. The formation of multiple covalent bonds to oxygen by uranium has been explained theoretically. The tendency of oxygen to delocalise its π electrons away from its highly-compact valence shell by forming π bonds with π electron acceptor metals accounts for the formation of metal oxygen bond, at least qualitatively. Complexes of the uranyl ion, UO₂²⁺, are of interest since they show seven-coordinate, pentagonal-bipyramidal geometry (Gatto et al., 2004). Due to the spectral properties (absorption and luminescence) and excited-state electron-transfer properties of the UO₂²⁺ ion, dioxouranium(VI) complexes have possible applications in solar energy conversion systems (Signorni and Dockal, 1996).

The Schiff base ligands obtained by condensation of various amines with salicylaldehyde/substituted salicylaldehydes are a class of ligands widely studied. Most studies are especially those of the first transition series. The Schiff base complexes with many transition metal ions, focused on complexes of the d-block
elements, have attracted considerable interest because of their growing importance as model molecules for biological systems such as oxygen carriers (Soliman and Mohamed, 2004; Syamal, 1978; Salam and Chowdhury, 2000, 2003).

The dioxouranium(VI) complexes with tetradeutate Schiff bases have been the subject of many investigations, while the oxouranium complexes with bi- or tridentate Schiff bases seem to be fewer in the literature (El-Tabl et al., 2002; Mandlik and Anwar, 2003). The tridentate dibasic Schiff base ligands behave as an ONO-donor ((El-Tabl et al., 2002) and react with the uranyl salts to form two types of complexes, depending on the molar ratio of the reactions.

However, little attention has been given to Schiff bases of ONS donor system. Co(II), Cu(II) and Zn(II) complexes of such Schiff base have been prepared and characterized (Soliman and Linert, 1999). Reaction modes of nickel(II) ion with the monodentate ligand diethylamine and the tridentate ligand 2-thiophenyl-1-hydoxyaldehydilidimino were studied (Elerman et al., 1996). Square planar nickel(II) complexes of ONS donor Schiff base ligands and triphenylphosphine were reported (Tamizh et al., 2009). Several oxovanadium(IV) complexes of Schiff bases derived from salicylaldehyde and 3-aminothiophenol were described (Syamal, 1978). Dioxouranium(VI) complexes of some aroylhazidines (benzoylhazine, salicyloylhazine, nicotinoylhazine) and their Schiff bases with acetone have been characterized where ligand acted as bidentate using NO-donor set (Chowdhury et al., 2008). The aim of this work is to study the behaviour, prepare and investigate the structure of the chelates dioxouranium(VI), UO$_2^{2+}$, complexes of the bidentate Schiff base ligands derived from o-hydroxyaldehyde or ketone, 2-hydroxy-1-naphthaldehyde and aniline, having as ON donor atoms.

**EXPERIMENTAL**

Uranyl nitrate hexahydrate, UO$_2$(NO$_3$)$_2$.6H$_2$O, was obtained from BDH Chemicals Ltd. Salicylaldehyde (Sal)/substitued salicylaldehyde, 5-chloro-salicylaldehyde ($^5$Cl-Sal), 5-bromo-salicylaldehyde ($^5$Br-Sal), 2-hydroxyacetphenone (HAP), 2-hydroxypropiophenone (HPP), 2-hydroxy-1-napthaldehyde (HNP), aniline (Ani) and other chemicals used were obtained from the M/S Aldrich Chemicals Co. Ltd.

**Preparation of Ligand LH**

Salicylaldehyde or substituted salicylaldehyde (Sal), 2-hydroxyacetphenone (HAP), 2-hydroxypropiophenone (HPP), 2-hydroxy-1-napthaldehyde (HNP) (10 mmol each) were reacted with aniline (Ani) in a round bottom flask, containing 50 mL ethanol, fitted with a reflux condenser and a silica gel guard tube. Each mixture was refluxed for 30 min with continuous stirring, using magnetic stirrer. The mixture was then cooled in ice-bath and kept over night where upon crystalline precipitate of respective ligands separated out. The product was filtered off, washed with ethanol and dried in vacuo over calcium chloride. Colour, yield and melting points of prepared Schiff base ligands are given in Table 1.
Table 1. Colour, yield and melting points of the prepared Schiff base ligands.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Ligands</th>
<th>Empirical formula</th>
<th>Physical state</th>
<th>Colour</th>
<th>% Yield</th>
<th>MP °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sal-AniH</td>
<td>HO(C₆H₄)CH=N(C₆H₅)</td>
<td>Solid</td>
<td>Pale green</td>
<td>80</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>BrSal- AniH</td>
<td>HO(C₆H₅Br)CH=N(C₆H₅)</td>
<td>Solid</td>
<td>Orange</td>
<td>85</td>
<td>112</td>
</tr>
<tr>
<td>3</td>
<td>ClSal- AniH</td>
<td>HO(C₆H₅Cl)CH=N(C₆H₅)</td>
<td>Solid</td>
<td>Orange</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>HAP- AniH</td>
<td>HO(C₆H₄)C(CH₃)=N(C₆H₅)</td>
<td>Liquid</td>
<td>Lt. yellow</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>5</td>
<td>HPP- AniH</td>
<td>HO(C₆H₄)C(C₂H₅)=N(C₆H₅)</td>
<td>Liquid</td>
<td>Lt. orange</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>6</td>
<td>HNP- AniH</td>
<td>HO(C₁₀H₆)CH=N(C₆H₅)</td>
<td>Solid</td>
<td>Deep orange</td>
<td>80</td>
<td>62</td>
</tr>
</tbody>
</table>

Preparation of complexes, UO₂L₂

4 mmol of respective ligand (L H) was taken in a round bottom flask containing 20 mL of mixed solvent of dichloromethane and ethanol by 10:1 (v/v). At reflux UO₂(NO₃)₂.6H₂O (2 mmol) was added to this with continuous stirring when colour changed immediately. The mixture was refluxed for one and half an hour on water bath when coloured precipitate came out. Mixture was then kept overnight. The precipitate was filtered in sintered funnel, washed with same solvent, and dried in vacuo over calcium chloride.

Physical Measurements

Melting point of the ligands and complexes were determined by an electrothermal melting point apparatus. UV-absorption spectra were run on a Shimadzu UV-visible recording spectrophotometer (model-160). Infrared spectra were recorded on KBr pellets with Perkin-Elmer infrared spectrophotometer (Model-883). Magnetic moments were determined by the Gouy method. Conductivity measurements were performed on Philips conductivity meter (model-WPA CM- 25) made by WPA, Saffron Walden, England. Metal analyses of the prepared complexes were done gravimetrically by oxine (Vogel, 1961). Some analytical and physical data of complexes are included in Table 2.

Table 2. Analytical and some physical data of the complexes.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Complexes</th>
<th>Colour</th>
<th>Yield (%)</th>
<th>MP °C</th>
<th>Metal content (%)</th>
<th>µeff Β.M.</th>
<th>Λm Ohm⁻¹ cm² mol⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UO₂(Sal-Ani)₂</td>
<td>Orange</td>
<td>80</td>
<td>250</td>
<td>34.49 (35.93)</td>
<td>Dia</td>
<td>1.4</td>
</tr>
<tr>
<td>2</td>
<td>UO₂(BrSal- Ani)₂</td>
<td>Orange</td>
<td>70</td>
<td>247</td>
<td>27.92(29.02)</td>
<td>Dia</td>
<td>4.8</td>
</tr>
<tr>
<td>3</td>
<td>UO₂(ClSal- Ani)₂</td>
<td>Deep yellow</td>
<td>75</td>
<td>250</td>
<td>31.83(32.55)</td>
<td>Dia</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>UO₂(HAP- Ani)₂</td>
<td>Deep orange</td>
<td>75</td>
<td>208</td>
<td>33.02(34.47)</td>
<td>Dia</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>UO₂(HPP- Ani)₂</td>
<td>Yellow</td>
<td>40</td>
<td>150</td>
<td>32.45(33.13)</td>
<td>Dia</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>UO₂(HNP- Ani)₂</td>
<td>Orange</td>
<td>85</td>
<td>270</td>
<td>30.86(31.21)</td>
<td>Dia</td>
<td>1.0</td>
</tr>
</tbody>
</table>

M.P. = melting point, dia=diamagnetic
*Values in parentheses indicate calculated values.
RESULTS AND DISCUSSION

$\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ reacts with the dinegatively-charged ligands in 1:2 ratio following the reaction scheme as shown in Figure 1 in slight warm conditions. As the ligand dissolved completely in general solvents, reaction proceed is shown by the colour change of the reaction mixture. Precipitates are formed upon cooling, which have been isolated and characterized by elemental analysis, IR and other conventional methods. All the complexes exhibit high melting points, indicating strong bonding between ligands upon complete deprotonation and neutral dioxouranium(VI) ion. All the complexes are stable at room temperature. The yields of the purified dioxouranium(VI) complexes for this general procedure are in the range 70-85\%. The complexes are slightly soluble in methanol, ethanol, acetone and chloroform, and fairly soluble in dimethylformamide and dimethylsulfoxide. The analytical data support 1:2 metal-ligand stoichiometries. The Schiff bases behave as dibasic tridentate ligands coordinating via the phenolic oxygen, azomethine nitrogen and thiolo sulphur atoms.

\[ \text{X} - \text{O} - \text{H} + \text{H}_2\text{N} - \text{R} \rightarrow \text{X} - \text{C} = \text{N} - \text{R} \]

\[ \text{o-hydroxy-aldehyde/ketone} \quad \text{Aniline} \]

\[ \text{R}=\text{H}, \text{CH}_3, \text{CH}_3\text{CH}_2 \]

\[ \text{X}=\text{Br}, \text{Cl}, 4, 5\text{-fused phenyl} \]

**Figure 1.** Reaction scheme and chemical structures of the ligands and the dioxouranium (VI) complexes.
IR Spectra

The infrared spectra of the present complexes are compared with those of corresponding ligand to determine the coordination sites of the ligands. The characteristic strong bands at 865-960 cm\(^{-1}\) and 785-880 cm\(^{-1}\) in the spectra of complexes have been assigned to \(\nu_{as}(O=U=O)\) and \(\nu_s(O=U=O)\), respectively (El-Sonbati et al., 2002; Yilmaz et al., 2008). The absorption bands appearing in the region 3205-3450 cm\(^{-1}\) are assigned to \(\nu_O-H\) (Chowdhury et al., 2008; Yilmaz et al., 2008). However, the O-H stretching frequency is dislocated to around 2583 cm\(^{-1}\) due to the hydrogen bridge OH....N=C (Figure 2) (Signorni, and Dockal, 1996; Chowdhury et al., 2008). These bands disappeared in the spectra of the complexes indicating coordination of phenolic oxygen atom (Soliman and Linert, 1999; Yilmaz et al., 2008). The negative shift of the \(\nu(C=N)\) in the spectra of the complexes indicates involvement of the azomethine nitrogen to coordination (Soliman and Linert, 1999). The absorption peaks appeared around 1550 cm\(^{-1}\) are attributable to \(\nu(C=C)\) (aromatic). The stretching frequencies of \(\nu_C-O\) (phenolic) and \(\nu_C-N\) (amino) appeared at 1261-1294 cm\(^{-1}\) and 1350-1381 cm\(^{-1}\), respectively, in the spectra of the present complexes (Soliman and Linert, 1999). The U-O and U-N modes have been assigned most tentatively at 500-516 and 409-419 cm\(^{-1}\), respectively (Salam et al., 1997; Chowdhury et al., 2008).

Table 3. Infrared spectral bands for the prepared complexes.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Ligands</th>
<th>(\nu_O-H)</th>
<th>(\nu_C=N)</th>
<th>(\nu_C-N)</th>
<th>(\nu_C-O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sal-AniH</td>
<td>3440 vs</td>
<td>1643 vs</td>
<td>1345 vs</td>
<td>1280 vs</td>
</tr>
<tr>
<td>2</td>
<td>BrSal-AniH</td>
<td>3450 vs</td>
<td>1632 vs</td>
<td>1340 vs</td>
<td>1270 vs</td>
</tr>
<tr>
<td>3</td>
<td>ClSal-AniH</td>
<td>3364 s</td>
<td>1645 vs</td>
<td>1381 vs</td>
<td>1266 vs</td>
</tr>
<tr>
<td>4</td>
<td>HAP-AniH</td>
<td>3205 vs</td>
<td>1638 vs</td>
<td>1377 vs</td>
<td>1270 vs</td>
</tr>
<tr>
<td>5</td>
<td>HPP-AniH</td>
<td>3250 vs</td>
<td>1600 vs</td>
<td>1379 vs</td>
<td>1258 vs</td>
</tr>
<tr>
<td>6</td>
<td>HNP-AniH</td>
<td>3240 vs</td>
<td>1618 vs</td>
<td>1347 s</td>
<td>1286 vs</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Complexes</th>
<th>(\nu_C=N)</th>
<th>(\nu_C-N)</th>
<th>(\nu_C-O)</th>
<th>(\nu_U=O=U)</th>
<th>(\nu_U-O)</th>
<th>(\nu_U-N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UO(_2)(Sal-Ani)(_2)</td>
<td>1640 vs</td>
<td>1381 vs</td>
<td>1283 vs</td>
<td>924 vs</td>
<td>515 vs</td>
<td>410 s</td>
</tr>
<tr>
<td>2</td>
<td>UO(_2)(BrSal-Ani)(_2)</td>
<td>1629 vs</td>
<td>1375 vs</td>
<td>1277 vs</td>
<td>923 vs</td>
<td>504 vs</td>
<td>409 vs</td>
</tr>
<tr>
<td>3</td>
<td>UO(_2)(ClSal-Ani)(_2)</td>
<td>1643 vs</td>
<td>1376 vs</td>
<td>1286 vs</td>
<td>930 vs</td>
<td>516 vs</td>
<td>412 vs</td>
</tr>
<tr>
<td>4</td>
<td>UO(_2)(HAP-Ani)(_2)</td>
<td>1596 vs</td>
<td>1377 vs</td>
<td>1279 vs</td>
<td>920 vs</td>
<td>500 vs</td>
<td>419 vs</td>
</tr>
<tr>
<td>5</td>
<td>UO(_2)(HPP-Ani)(_2)</td>
<td>1601 vs</td>
<td>1371 vs</td>
<td>1261 vs</td>
<td>917 vs</td>
<td>509 vs</td>
<td>414 vs</td>
</tr>
<tr>
<td>6</td>
<td>UO(_2)(HNP-Ani)(_2)</td>
<td>1628 vs</td>
<td>1350 s</td>
<td>1294 vs</td>
<td>924 vs</td>
<td>512 s</td>
<td>418 s</td>
</tr>
</tbody>
</table>

**Figure 2.** Hydrogen bonding in ligand molecule.
Electronic Spectra

The electronic spectrum of the ligand (LH) in ethanol shows absorption bands at 267-381 nm. The bands appearing at the UV region are attributable to π - π* transitions associated with the azomethine chromophores. The bands at higher energy arise from π - π* transitions within the phenyl rings. The absorption bands of the complex are shifted to longer wavelength compared to those of the ligand. A moderately intensive band observed in the range of 330–395 nm is attributable to the n - π* transitions of the complex. However, the typical band of UO₂²⁺ expected around 400 nm seems to be overlapped by fairly strong ligand-to-metal charge-transfer bands. These charge-transfer transitions probably occur from the n - π* orbitals of the Schiff base to the f-orbitals of uranium (Elerman et al., 1996). Electronic spectral bands for prepared complexes are given in Table-4.

Table 4. Electronic spectral bands for the prepared ligands and their complexes.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Ligands</th>
<th>Electronic spectra (nm)</th>
<th>Complexes</th>
<th>Electronic spectra (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sal-AniH</td>
<td>329, 306, 271</td>
<td>UO₂(Sal-Ani)₂</td>
<td>400, 314, 305, 271</td>
</tr>
<tr>
<td>2</td>
<td>BrSal- AniH</td>
<td>321, 304, 271</td>
<td>UO₂(BrSal- Ani)₂</td>
<td>348, 337, 319, 308, 272</td>
</tr>
<tr>
<td>3</td>
<td>ClSal- AniH</td>
<td>318, 304, 271</td>
<td>UO₂(ClSal- Ani)₂</td>
<td>350, 339, 306, 272</td>
</tr>
<tr>
<td>5</td>
<td>HPP- AniH</td>
<td>313, 305, 267</td>
<td>UO₂(HPP- Ani)₂</td>
<td>344, 316, 308, 270</td>
</tr>
<tr>
<td>6</td>
<td>HNP- AniH</td>
<td>381, 317, 304, 269</td>
<td>UO₂(HNP- Ani)₂</td>
<td>438, 381, 319, 309, 270</td>
</tr>
</tbody>
</table>

Magnetic and Conductivity Measurement

The magnetic susceptibility of the complexes was found to be negative, indicating the complexes to be diamagnetic as expected for f₆, 5f₆d₆s⁶, U(VI) complexes possessing its 6+ oxidation state. The magnetic measurements of the dioxouranium(VI) complexes are independent of field strength and temperature and the ground states of dioxouranium(VI) compounds contain no unpaired electrons. This is consistent with diamagnetic behaviour expected for the U(VI) electronic spectra of complexes.

The molar conductance values (Table 2), measured in DMF (10⁻³ M) solution of the complex, gave a value of 0-4.8 ohm⁻¹ cm² mol⁻¹ which indicates their non-electrolytic behaviour as tentative for the complexes (El-Sonbati et al., 2002).
CONCLUSION

As a general conclusion, the prepared Schiff bases behave as a monobasic ligand in 1:2 complexes with bidentate ON donors derived from the phenolic oxygen and the azomethine nitrogen. Therefore, on the basis of elemental analyses and analytical data, an octahedral geometry has been proposed for the \([\text{UO}_2\text{L}_2]\) type complexes as in Figure 3. However, it is difficult to suggest the exact geometry of the present complexes without crystal structural evidence.

Figure 3. Proposed octahedral geometry for the prepared complexes.

REFERENCES


Incidence of Indoor Airborne Fungi at the Central Library of Rajshahi University and Their Relation to Allergy Symptoms

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ABSTRACT

The indoor air sampling was conducted at the Central Library of Rajshahi University by settling plate technique during November 2006 to April 2007 using Potato dextrose agar, Czapek’s and Sabouraud’s media. Total of 4,613 colonies of airborne fungi were trapped and 11 genera were identified. The most frequently isolated genera were Alternaria, Aspergillus, Curvularia, Fusarium, Penicillium and Rhizopus. Percentages of the six dominant genera were recorded as 29.25, 14.55, 13.64, 10.79, 7.48 and 6.43%; 26.83, 9.58, 14.25, 8.77, 8.19 and 7.38%; and 25.05, 12.98, 9.32, 7.25, 5.85 and 14.63% on PDA, Czapek’s and Sabouraud’s media, respectively. The incidence of airborne fungi significantly \((p=0.05)\) varied with floor and the highest incidence was recorded at ground floor, followed by 1st floor and the lowest in 2nd floor. Among the 11 identified genera, Aspergillus, Fusarium, Mucor and Penicillium showed positive results in hemolytic activity test. The incidence of airborne fungi was correlated with allergy symptoms of employees, students and researchers, showing the highest peak in April, 2007.

Key words: Indoor airborne fungi, Percentage contribution, Room condition, Hemolytic activity, Allergy symptoms
INTRODUCTION

Air quality in indoor environment is being recognized as an important issue in public health (Li and Kendrick, 1995a). As many as 30% of buildings worldwide have indoor air quality complaint (WHO, 1983) and in the United States, as many as 40% of homes and 115,000 schools have health problems, linking to poor indoor air quality (Spengler et al., 1994). Fungal exposures have been documented to cause allergic disease (1 in 4 people worldwide), toxicoses, irritation and infections (Barge, 1990; Chao et al., 2002) and blamed for building related symptoms (Harrison et al., 1992). In developed countries, the majority of the people spend more than 90% of their time indoors, and thus experience long exposure to common airborne pollutants which have potentially adverse health effects. To systematically evaluate the relationship between airborne fungi and adverse health effect, the fungal types and their relative frequencies in indoor airs need to be known (Shelton et al., 2002). Sources for indoor airborne fungi can be outdoor air and indoor reservoirs (Berge, 1995; Li and Kendrick, 1996). Although outdoor fungi cannot go easily inside the large buildings with complex ventilation systems, the outdoor aerosol still may dominate indoors (Burge et al., 2000). Accumulated dust and room materials, viz., wallpaper, carpeting, ventilation duct surfaces can also become bioaerosol sources if water content can support the growth of microorganisms (Barge, 1990; Berge, 1995; Burge et al., 2000; Stolwijk, 1991).

Information obtained from fungal air samples can assist in medical evaluation, determination of remedial procedures and assessment of health hazards and can be useful in proactive indoor air quality monitoring. However, there are no government or industry standards that specify acceptable concentrations of indoor airborne fungi and only limited information is available on airborne fungal types and their prevalence inside the buildings. So, the present attempt has been undertaken to assess the incidence of indoor airborne fungi at the Central Library of Rajshahi University and their possible relations with the environmental factors like temperature and relative humidity. The hemolytic activity of identified genera was tested and the research was also expanded to determine the relations of airborne fungi with library environment and occupants/workers.

MATERIALS AND METHODS

Sampling

The indoor air sampling was conducted at different sites of the Central Library of Rajshahi University Rajshahi during November 2006 to April 2007. Using Czapek’s, Potato Dextrose Agar (PDA) and Sabouraud’s media, air samples were collected from near occupants breathing zone (approx. 1 m above ground), following settling plate technique. At each sampling site, a total of 36 culture plates [4 samplings per month × 3 plates for each culture medium (triplicate) × 3 culture media] were collected per month. Room temperature and relative humidity (RH) were recorded at each sample site. The presence of dampness, visible fungi and cleanliness of the sample areas were also noted during sampling.
**Enumeration and identification of airborne fungi**

The Petridishes (9 cm diam) containing media were exposed for fifteen minutes during sampling and incubated at room temperature for four to seven days. This was followed by the counting of the fungal colonies on the culture plate. Sub-cultures of the fungi recovered from air sampling were maintained on slant cultures for various periods to identify fungi, induction of sporulation of sterile mycelia etc. Identification of the fungi was made by visual (colony morphology) and microscopic observation. Identification up to generic level was done with the help of standard mycological literature (Gilman, 1957; Booth, 1971; Subramanian, 1971; Ellis, 1971; Alexopoulos and Mims, 1979). Sub-cultures of the fungal mycelia which failed to sporulate up to the end of one month were designated as sterile mycelia.

Details regarding the qualitative nature of the mycoflora, their incidence, abundance and percentage contribution were recorded. The percentage contribution of each genus was calculated on the basis of the number of colonies of a genus against the total number of colonies of all recorded genera during the entire six months of sampling period.

**Hemolytic activity test**

For hemolytic activity test, blood agar medium (5% cow blood) was used. Spore suspension (10^6/ml) of each trapped genus was spread on the plate containing blood agar and kept in an incubator at 30˚C for two to three days. Fungal colonies, developing from incubated spore suspension, showing zone of clearing around them are considered as hemolytic-positive.

**Questionnaires**

For determination of relationships of indoor airborne fungi and allergy symptoms, employees, students and researchers were asked to complete initial questionnaires during the observation period of six months. The questionnaires included questions about personal data and health complications. The options for allergy symptoms were indicated by allergist as plugging, itchy, sneezing or running nose; itchy, watery, swelling and redness of eye; throat sore, swelling; chest tightness, cough and difficulties of breathing.

**Statistical analyses**

The experiment was conducted by using a completely randomized design with three replications. All data were analyzed by F-test. Results of all analyses were judged for significance at 5% level.
RESULTS AND DISCUSSION

A total of 4,613 viable fungal colonies were trapped from indoor atmosphere of the Central Library of Rajshahi University using Czapek’s, Potato dextrose agar and Sabouraud’s media during November 2006 to April 2007. Among them, 4039 colonies of fungi were identified, 524 colonies were sterile and 50 colonies were unidentified. The isolated fungi were assigned to 11 genera, belonging to Phycomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes. Among the three media tested, PDA was most favorable for growth and development of identified fungi and the maximum sterile mycelia were recorded on Czapek’s medium. Monthly variation in total fungal colonies with respect to temperature and average relative humidity was observed on PDA, Czapek’s and Sabouraud’s media during the investigation (Fig.1). The highest temperature (31.27°C) and low relative humidity (45.97%) were recorded in April which was associated with the highest number of fungi. The lowest temperature (15.35°C) and moderate humidity (51.30%) were recorded in January that were related with the lowest number of fungi in the indoor atmosphere of the Central Library. Thus, temperature seems to be positively correlated with the incidence of airborne fungi and has an effect on increasing the number of airborne fungi. Chao et al., (2002) observed that the temperature is the critical environmental factor which control microbial growth in indoors and total airborne fungal concentration is positively correlated with relative humidity (RH) at below 30% and above 40%. Wright et al., (1969) also opined that the prevalence of airborne mycoflora was intimately related with prevalent climatic conditions including temperature and relative humidity.
Figure 1. Monthly occurrence of total indoor aerial fungi with respect to some environmental factors, as recorded on PDA, Czapek’s and Sabouraud’s media.
Incidence of indoor airborne fungi in different months

During the observation of six months, a total of 2178, 1368 and 1641 colonies were counted respectively on PDA, Czapek’s and Sabouraud’s media. The highest number of fungal colonies was recorded in the month of April and the lowest was exhibited in January (Fig. 2). Chao et al., (2002) reported that total number of airborne fungi decreased throughout the summer and winter, and then began to increase in April. Shelton et al., (2002) also reported that the sizes of fungal populations varied significantly by seasons. The present observations support the above findings.

Among the identified 11 genera, Penicillium was the most dominating genus on all media used. The lowest genera were Colletotrichum (1.33% on PDA), Trichoderma (0.66% on Czapek’s) and Cladosporium (1.71% on Sabouraud’s) during the six months of observation. The most frequently isolated genera were Alternaria, Aspergillus, Curvularia, Fusarium Penicillium and Rhizopus and their percentages of occurrence were 29.25, 14.55, 13.64, 10.79, 7.48 and 6.43%; 26.83, 9.58, 14.25, 8.77, 8.19 and 7.38%; and 25.05, 12.98, 9.32, 7.25, 5.85 and 14.63% on PDA, Czapek’s and Sabouraud’s media, respectively. Colonies of sterile mycelia and unidentified fungi covered 7.67 and 0.64% of abundance on PDA; 13.16 and 1.24% on Czapek’s; and 10.79 and 1.16% on Sabouraud’s media, respectively.
Figure 2. Monthly incidence of airborne fungi as recorded in PDA, Czapek’s and Sabouraud’s media during November 06 to April 07.
Incidence of indoor airborne fungi at different floors of Central Library

Total numbers of viable airborne fungal colonies were counted as 851, 692 and 635 on PDA; 527, 434 and 407 on Czapek’s; and 626, 534 and 481 on Sabouraud’s medium and these were collected from the indoor atmosphere of ground floor, 1st floor and 2nd floor of the Central Library, respectively, during the observation of six months from November 2006 to April 2007 (Fig. 3). The incidence of airborne fungi significantly (P=0.05) varied at different floors. The highest incidence of airborne fungi was recorded at ground floor, followed by 1st floor and the lowest in 2nd floor. Szam et al., (1981) observed the vertical development of the airborne mycoflora and reported that the highest incidence of mycoflora present at ground floor but their occurrence decreased at the upper stories. The present result completely corroborates the findings of Szam et al., (1981).

The genera *Alternaria, Aspergillus, Curvularia, Fusarium, Penicillium* and *Rhizopus* were recorded as the most frequently occurring genera in air of floors of the Central Library. Begum et al., (2007) reported that, *Alternaria, Aspergillus, Candida, Cladosporium, Curvularia, Fusarium, Gloeosporium, Neurospora, Penicillium* and *Sporobolomyces* were the most frequent genera in the air of Rajshahi Metropolitan City. Uddin (2005) reported that *Penicillium* and *Aspergillus* are the most dominant fungi followed by *Curvularia* and *Cladosporium*. Albuquerque et al., (2004) studied the airborne fungi of Brazil and reported that *Absidia, Alternaria, Aspergillus, Cladosporium, Curvularia, Dreschleria, Fusarium, mycelial sterila* (the fungi don’t producing any spores), *Cladosporium, Penicillium* and *Penicillium and Rhizopus* were predominant fungi. Shelton et al., (2002) reported that non sporulating fungi are prevalent fungi in the air of U.S.A.

Among all fungi identified, *Penicillium* was the most prevalent genus as recorded at all floors and all media which covered 22.68, 32.95 and 34.02% of total counts on PDA; 28.27, 28.57 and 23.10% on Czapek’s medium and 20.93, 28.09 and 27.03% on Sabouraud’s medium at ground floor, 1st floor and 2nd floor of Central Library, respectively, during six months of observations. The next dominating genera were *Alternaria, Aspergills, Curvularia, Fusarium* and *Rhizopus* which covered 4.10 to 29.26% on PDA; 5.76 to 15.56% on Czapek’s; and 4.15 to 23.00% on Sabouraud’s media, respectively. The highest percentage of sterile mycelia was recorded at 2nd floor which covered 8.9, 18.8 and 13.51% on PDA, Czapek’s and Saboraud’s media, respectively.
Figure 3. Incidence of different airborne fungi as recorded on PDA, Czapek’s and Sabouraud’s media during 6 months of observation (November 2006 to April 2007) at different sites of central library of Rajshahi university.
Hemolytic activity test of isolated indoor airborne fungi

Identified 11 genera, viz., Alternaria, Aspergillus, Cladosporium, Colletotrichum, Curvularia, Fusarium, Mucor, Oidiodendron, Penicillium, Rhizopus and Trichoderma were tested for the hemolytic activity on blood agar medium (5% cow blood). Only eight genera such as Aspergillus, Fusarium, Mucor and Penicillium showed positive result. So, they are hemolytic in nature and may be pathogenic. Some microorganisms secrete hemolysins that lyse red blood cells which contribute to their pathogenicity. Hemolytic strain is more virulent than non-hemolytic strains of the same species. A large number of hemolytic bacteria have been demonstrated in the floor dust of the hospital wards (Coriell, 1968). Khan and Ali (1984) reported that hospital wards are highly contaminated with certain hemolytic bacteria associated with certain diseases of the respiratory tract.

Incidence of airborne fungi and their relation to allergy symptoms

In the present investigation, a total of 1,200 employees, students and researchers participated in the observation of six months. Their age ranged from 20 to 55 and they had suffered from several types of health complications. The health complications generally increases during March to April. In room conditions, dampness is usually observed in March to April; visible fungi especially moulds appear in maximum Walls, materials, a few number of books and the floors are cleaned every day by ordinary water. This insufficient room management may increase the incidence of indoor airborne fungi. Environmental variables have been associated with perceptions of health of office occupants (Chao et al., 2002). Significantly greater spore counts and higher prevalence of allergic symptoms were found in damp residences (Li and Kendrick, 1995b). Allergic problems may occur at high concentrations of airborne fungal spores (van Bronswijk et al., 1986). From the analyses of survey data, it was found that the highest % of allergy symptoms was noted in April which was positively correlated with the incidence of indoor airborne fungi. Total spores may have an impact on increasing incidence of allergy symptoms; in that case, some of the indoor fungi in the total spores had adverse effects on human health. (Li et al., 2002).
Table 1. Evaluation on age range, profession, health complication, room condition, and incidence of airborne fungi with % of allergy during November 2006 to April 2007

<table>
<thead>
<tr>
<th>Months &amp; Week</th>
<th>Age range</th>
<th>Profession</th>
<th>Health complication</th>
<th>Room condition</th>
<th>Incidence of airborne fungi on</th>
<th>% of allergy affected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dampness</td>
<td>Visible fungi</td>
<td>Cleanness</td>
</tr>
<tr>
<td>November</td>
<td>1st</td>
<td>20-55</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Cough</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>20-45</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis.</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>25-55</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Cough</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4th</td>
<td>20-45</td>
<td>Emp., St.&amp; Res.</td>
<td>Cough, Conj.</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>December</td>
<td>1st</td>
<td>20-55</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Cough</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>20-45</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Headache</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>25-55</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Cough</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4th</td>
<td>20-45</td>
<td>Emp., St.&amp; Res.</td>
<td>Cough, Conj.</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>January</td>
<td>1st</td>
<td>20-55</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Cough</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>20-45</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Headache</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>25-55</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Cough</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4th</td>
<td>20-45</td>
<td>Emp., St.&amp; Res.</td>
<td>Cough, Conj.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>February</td>
<td>1st</td>
<td>20-55</td>
<td>Emp., St.&amp; Res.</td>
<td>Cough, Diff. breathing</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>20-45</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Cough</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>25-55</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis.</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4th</td>
<td>20-45</td>
<td>Emp., St.&amp; Res.</td>
<td>Cough, Skin dis.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>March</td>
<td>1st</td>
<td>20-55</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Cough, Conj.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>20-45</td>
<td>Emp., St.&amp; Res.</td>
<td>Cough, Skin dis.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>25-55</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Cough, Conj., Th. xoar, Headache</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4th</td>
<td>20-45</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Cough, Conj., Th. xoar, Headache</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>April</td>
<td>1st</td>
<td>20-55</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Cough, Conj.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>20-45</td>
<td>Emp., St.&amp; Res.</td>
<td>Cough, Skin dis., Headache</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>25-55</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Cough, Conj., Th. xoar, Headache</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4th</td>
<td>20-45</td>
<td>Emp., St.&amp; Res.</td>
<td>Skin dis., Cough, Conj., Th. xoar, Headache</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Emp = employees, St = students, Res = researchers, Dis = disease, Conj = conjitivites, Th=Throat
CONCLUSION

Estimation of fungal exposures is of increasing importance in assessing indoor air quality. Microbiological air contamination is caused by ventilation systems of large buildings and it may play a role on adverse health. In this case, disinfectant can be used in swiping which may reduce airborne fungi. So, in this circumstance, the present investigation will help in making future sanitation program to maintain libraries or large buildings to protect human health.

REFERENCES

Production of Propionic Acid for Antifungal Activity by Calcium alginate Immobilization of Propionibacterium acidipropionici TISTR 442 Using Whey as Substrate

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ABSTRACT

The improvement of propionic acid production, as fermenting by calcium alginate immobilized cells of Propionibacterium acidipropionici TISTR 442 and using whey as substrate was investigated. The highest amount of propionic acid was produced by adjusting the distance between the tip of the tube and the surface of CaCl₂ solution to be 4 - 6 cm and the flow rate of the gel solution to be 7 ml/min. Fermentation by immobilized cells in 2-l fermentors using 1% CaCO₃ and 5 N KOH to control the pH at 6.5 gave the highest amount of propionic acid 29.24 g/l and consistent potential to recycle 2 rounds of fermentation by producing the total amount of 15.85±0.25 g/l and 13.39±0.25 g/l propionic acid from Batch 1 and Batch 2 fermentation, respectively. Which one amount of propionic acid was higher than that from free cells in 2-l fermentors at 216 h of the fermentation time. The fermented propionic acid as well as the commercial propionic acid from chemical processes were able to inhibit the growth of the fungal tested.

Key words: Immobilization, Whey, Propionibacterium acidipropionici, Antifungal activity
INTRODUCTION

The demand of consumers for natural preservatives has caused an interest in using the preserving capacity of bacteria, naturally occurring in food and feeds (Lind et al., 2005). Propionic acid, the biopreservative produced by Propionibacterium sp., is able to inhibit the growth of molds, bacteria and dairy-spoilage yeasts such as Zygosaccharomyces bailii and Candida sp. (Schwenninger and Meile, 2004). In addition, propionic acid and its salts are used as raw materials in perfume and plastic industries (Goswami and Srivastava, 2000).

Commercial production of propionic acid is chiefly carried out by chemical synthesis which is an expensive recovery process and harmful chemicals can occur. If higher yields of propionic acid are obtained, production by fermentation will become economically competitive and will offer several advantages over chemical synthesis (Himmi et al., 2000). The advantages include the ability to label the product as a “natural preservative” which responds to the consumer’s demands and the fermentation process that harmful chemicals will not be released.

Several processes have been patented for producing propionic acid by fermentation. Batch method using a variety of substrates produced 1 to 3% propionic acid in 7-14 days (Playne, 1985; Blanc and Goma, 1989) and other processes such as fed-batch and continuous system of the fermentation have been used to improve the yield of propionic acid. However, the major factor that limits the production of propionic acid during fermentation is the end-product inhibition by acid (Woskow and Glatz, 1991). Therefore, immobilized-cell reactors have been developed to protect cells from the end-product inhibition which could enhance the yield and productivity of the fermentation.

Immersed cells have been used for production of organic acids, amino acids, antibiotics, enzymes and other compounds. By using immobilized cells, the process can be controlled much easier than using a batch system of free cells. In addition, immobilized cells are more stable than free biomass cells and the product separation is easier (Ates et al., 2002).

In this investigation, the fermentation by immobilized cells of P. acidipropionici TISTR 442, using whey as substrate, was studied in order to enhance the yield of propionic acid by reusing the immobilized cells (Jianlong, 2000) and using whey, the inexpensive substrate which was the by-product from dairy industry, in order to increase its commercial value.

MATERIALS AND METHODS

Microorganisms

Propionibacterium acidipropionici TISTR 442, Aspergillus flavus TISTR 3365, A. niger TISTR 3012, A. oryzae TISTR 3014, Rhizopus sp. TISTR 3024, Penicillum sp. TISTR 3046, Fusarium moniliforme TISTR 3175, Trichoderma sp. TISTR 3327, Candida albican TISTR 5239, C. sake TISTR 5143, Pichia membranefacien TISTR 5072, Saccharomyces cerevesiae TISTR 5017, Zygosaccharomyces rouxii TISTR 5044, Kluyveromyces marxianus TISTR 5163 and
Rhodotorula glutinis TISTR 5159 were from TISTR Culture Collection Bangkok, Mircen, Thailand.

Propionibacterium acidipropionici TISTR 442 was grown statically on de Man Rogosa and Sharpe (MRS) broth at 30˚C for 48 h and an inoculum of 5% (v/v) was used.

**Culture conditions**

5% (v/v) of the seed culture was transferred to a 250-ml flask containing 175-ml of each of the following six cultures;

- MRS broth (HiMedia Laboratories Pvt. Ltd. India).
- MRS broth plus 1% CaCO$_3$.
- Modified broth (modified from Gu et al., 1998; Goswami and Srivastava, 2000) containing 10 g/l yeast extract, 40 g/l lactose, 5 g/l trypticase soy broth, 0.25 g/l K$_2$HPO$_4$, 0.2 g/l MgSO$_4$.7H$_2$O and 0.05 g/l MnSO$_4$.4H$_2$O.
- Modified broth containing 10 g/l yeast extract, 40 g/l lactose, 5 g/l trypticase soy broth, 0.25 g/l K$_2$HPO$_4$, 0.2 g/l MgSO$_4$.7H$_2$O, 0.05 g/l MnSO$_4$.4H$_2$O and 1% CaCO$_3$.
- Whey (from Minor Dairy’s Factory, Thailand) broth dissolved with 10 g/l yeast extract, 0.25 g/l K$_2$HPO$_4$, 0.2 g/l MgSO$_4$.7H$_2$O and 0.05 g/l MnSO$_4$.4H$_2$O.
- Whey broth dissolved with 10 g/l yeast extract, 0.25 g/l K$_2$HPO$_4$, 0.2 g/l MgSO$_4$.7H$_2$O, 0.05 g/l MnSO$_4$.4H$_2$O and 1% CaCO$_3$.

Before inoculation of the seed culture, pH of the medium was adjusted to 6.5-7, then heat-sterilized at 121˚C and 15 psi for 15 min (without lactose). Lactose was autoclaved separately and added aseptically to the medium. Samples were taken from cultures every 24 h until the 336 h to analyse for propionic acid by high performance liquid chromatography (HPLC: SHIMADZU C-R7 AE PLUS, JAPAN)

**Cell Immobilization**

The seed culture (final absorbant $\sim$ 0.5 with a total population of $7.50 \times 10^6$ cfu ml$^{-1}$) was centrifuged at 10,000 g for 10 min and the spun broth was decanted. Pelleted cells were resuspended in sterile 0.85% NaCl solution and re-centrifuged. After the NaCl solution was decanted, the pelleted cells were mixed with sterile 0.85% NaCl solution and 2% sterile sodium alginate at a volumetric ratio of 10:3:20 (cells:saline:alginate). The mixture was extruded with a peristaltic pump through the tube into 0.1 M CaCl$_2$ solution to form beads. The beads were suspended in 0.1 M CaCl$_2$ solution at 4˚C for 4 hours and were washed thoroughly twice with sterile distilled water before being used.

**Distance optimization**

The immobilized cells with various distances of 2, 4, 6, 8, 5, 10, 15 and 20 cm from the end of the tube to the surface of CaCl$_2$ solution were investigated for gel formation and the optimal distance was selected for further studies.
Flow rates optimization

Immobilized cells on the selected distance were experimented with various flow rates of the gel solution at 5, 6 and 7 ml/min, and the optimal flow rate was selected.

Fermentation and cells recycling

The content of the 2-l fermentor containing 1.4 liters of selected medium was maintained at a constant pH of 6.5, 30˚C and 100 rpm agitation. Samples were taken from the culture every 24 h until at 216 h, when the medium was then drained out of the fermentor and a fresh medium was added to the beads before the repeated batch fermentation was started.

Antifungal activity

Propionic acid was extracted from the culture (Gu et al., 1998) and a comparison test with commercial propionic acid for antifungal effect by agar disc diffusion method was then performed (Faria et al., 2006). Concentration of 10^6 spores/ml solutions of tasted mold and 10^6 cells/ml solutions of yeast were prepared. One hundred μl of each of the solutions were pipetted and spread on sterile petri dishes containing potato-dextrose-agar (PDA) medium. One hundred μl of the fermented and the commercial propionic acid were then pipetted onto sterile Whatmann No. 1 paper discs which were in turn placed on the center of the PDA agar plates inoculated with a fresh fungal spores or cell suspension (one microorganism per Petri dish). The Petri dishes were then incubated at 30˚C for 4 days. The degree of inhibition was measured as the inhibited growth area of the Petri dish. Distilled water instead of the two types of propionic acid was used as a control.

Assay methods

The supernatants of the samples which had been 20 min at 5,000 g centrifuged samples were detected by HPLC and the propionic acid was quantified by filtering the samples through 0.45μm cellulose membranes in an Inertsil C8-3 column. The wave length of the UV detector was 210 nm and the flow rate was 0.1 mm/min. The total sugar was determined by the Dubois’s process (Dubois et al., 1956).

Statistical analyses

All fermentation experiments were run in triplicate. Statistical analyses were performed by Duncan’s New Multiple Range Test while antifungal activity was performed by Pair Sample T-Test.
RESULTS AND DISCUSSION

Typical medium and acid production

Propionic acid production by Propionibacterium acidipropionici TISTR 442 grown in 6 cultures are shown in Figure 1. In whey broth added with 1% CaCO$_3$, P. acidipropionici TISTR 442 produced the highest amount of propionic acid, 14.26±0.16 g/l (Table 1) with the reason that whey is composed of nutrient supplement and high lactose which are necessary for propionic acid production. These results were similar to those of Yang et al., (1994) who studied the continuous production of propionate from whey lactose by P. acidipropionici ATCC 4875, immobilized in a fibrous bed bioreactor, and it was found that 2% (w/v) propionic acid was obtained from 4.2% lactose fed at a retention time of 35-45 hours and the propionic acid yield was 46% (w/v) from lactose.

It was also found that in the comparisons of the media added with 1% CaCO$_3$ and without CaCO$_3$, the media added with 1% CaCO$_3$ had more acid occurred than that without CaCO$_3$ with the reason that CaCO$_3$ can reduce the acidity of the accumulated propionic acid in broth, therefore, free cells of P. acidipropionici could grow and produce propionic acid continuously. Because of having higher productivity, whey broth added with 1% CaCO$_3$ was selected for fermentation in the next step. Calculations of the propionic acid used for statistical analyses on various broths are shown in Table 1. The concentration of propionic acid from whey broth added with 1% CaCO$_3$ was significantly higher than that of the other broths at 95% confidence level.

In addition, whey contains high lactose which results in more products due to the reason that lactose was fermented to become propionic acid by the propionibacteria. Using whey as substrate reduced the cost and increased the values of the by-products from dairy industry.
Figure 1. Propionic acid production by free cells of *P. acidipropionici* TISTR 442 on various broths at 336 h:

(▲) whey broth added with 1% CaCO$_3$, (■) modified broth added with 1% CaCO$_3$, (●) MRS broth added with 1% CaCO$_3$, (Δ) whey broth, (❑) modified broth, (❍) MRS broth

Table 1. Comparisons of various broths for propionic acid production and for total sugar consumption at 336 h

<table>
<thead>
<tr>
<th>Type of broth</th>
<th>Propionic acid (g/l)</th>
<th>Total sugar consumption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRS</td>
<td>4.75$^{d} \pm 0.25$</td>
<td>74.13</td>
</tr>
<tr>
<td>MRS+CaCO$_3$</td>
<td>12.52$^{b} \pm 0.41$</td>
<td>94.23</td>
</tr>
<tr>
<td>modified</td>
<td>5.73$^{c} \pm 0.08$</td>
<td>75.22</td>
</tr>
<tr>
<td>modified+CaCO$_3$</td>
<td>13.0$^{b} \pm 0.33$</td>
<td>98.79</td>
</tr>
<tr>
<td>whey</td>
<td>6.04$^{c} \pm 0.16$</td>
<td>75.29</td>
</tr>
<tr>
<td>whey+CaCO$_3$</td>
<td>14.26$^{a} \pm 0.16$</td>
<td>98.96</td>
</tr>
</tbody>
</table>

Batch fermentation of free cells was performed in 250-ml flasks with a 175-ml working volume at a static state of 30°C for 336 h. Data are presented as means of three replications $\pm$ standard deviations.
Batch fermentation by free cells of *P. acidipropionici* TISTR 442 in 2-l flasks and 2-l fermentors

Propionic acid production and total sugar consumption by free cells of *P. acidipropionici* TISTR 442 in 2-l flasks and 2-l fermentors are shown in Figure 2. After 216 h fermentation, the acid in the fermentors was nearly in a static level due to the lack of total sugar. However, slightly slow cell growth in the flasks and the total sugar are remained and the propionic acid was therefore produced continuously in the flasks. In the 2-l flasks, free cells gave 14.33 ± 0.25 g/l propionic acid at 316 h. However, more propionic acid (15.16 ± 0.24 g/l) in the 2-l fermentors was produced in a more rapid time (216 h) with a pH of 6.5 maintained with KOH. Agitation was done in order to mix the cultures and the microorganisms occurred through fermentation and made up an optimal condition for propionic acid production. Champagne et al., (1989) studied about whey fermentation by immobilized cells of *P. shermanii* and found that agitation increased propionic acid fermentation rates but lowered the ratio of propionic acid to acetic acid.

![Figure 2](image-url)  
**Figure 2.** Comparisons of propionic acid production and total sugar consumptions in 2-l fermentors and 2-l flasks by free cells of *P. acidipropionici* TISTR 442: (▲) propionic acid in 2-l flasks, (●) propionic acid in 2-l fermentors, (∆) total sugar in 2-l flasks, (〇) total sugar in 2-l fermentors
Optimal conditions of gel formation

The conditions of gel formation were studied by varying the distance from the end of the tube to the surface of the CaCl₂ solution in order to determine the optimal values. Propionic acid productions by immobilized cells in the distances of 4-6 cm from the end of the tube to the surface of CaCl₂ solution were significantly higher than those at other distances at 95% confidence level (Table 2). The propionic acid production was significantly decreased when the distance increased.

Table 2. Comparisons of distances from the end of the tube to the surface of CaCl₂ solution on immobilization for propionic acid production and for total sugar consumption

<table>
<thead>
<tr>
<th>Distance (cm.)</th>
<th>Propionic acid (g/l)</th>
<th>Total sugar consumption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>17.68bc ± 0.49</td>
<td>98.95</td>
</tr>
<tr>
<td>4</td>
<td>18.37a ± 0.16</td>
<td>99.2</td>
</tr>
<tr>
<td>5</td>
<td>18.53a ± 0.15</td>
<td>99.26</td>
</tr>
<tr>
<td>6</td>
<td>18.49a ± 0.32</td>
<td>99.22</td>
</tr>
<tr>
<td>8</td>
<td>18.23ab ± 0.16</td>
<td>98.93</td>
</tr>
<tr>
<td>10</td>
<td>17.38c ± 0.24</td>
<td>98.9</td>
</tr>
<tr>
<td>15</td>
<td>17.20cd ± 0.09</td>
<td>98.87</td>
</tr>
<tr>
<td>20</td>
<td>16.67d ± 0.48</td>
<td>98.74</td>
</tr>
</tbody>
</table>

Batch fermentation of immobilized cells was performed in 250-ml flasks with a 175-ml working volume at a static state of 30°C for 336 h. Data are presented as means of three replications ± standard deviations.

Fixing flow rates of gel solutions at 5, 6 and 7 ml/min produced 18.97±0.25, 19.19±0.08 and 19.22±0.15 g/l propionic acid, respectively (Table 3). The highest propionic acid was produced by controlling the flow rate of the gel solution to be 6 or 7 ml/min. Although the productions of propionic acid at the 6 and 7 ml/min flow rates of the gel solution were not significantly different at 95% confidence level, the 7 ml/min flow rate which was faster saved up time in the immobilization process. Therefore, the fixing of flow rate of the gel solution at 7 ml/min was selected as the optimal condition of gel formation in further investigations.
Table 3. Comparisons of flow rates of gel solutions on immobilization for propionic acid production and for total sugar consumption

<table>
<thead>
<tr>
<th>Flow rate (ml/min)</th>
<th>Propionic acid (g/l)</th>
<th>Total sugar consumption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>18.97b ± 0.25</td>
<td>97.54</td>
</tr>
<tr>
<td>6</td>
<td>19.19a ± 0.08</td>
<td>97.66</td>
</tr>
<tr>
<td>7</td>
<td>19.22a ± 0.15</td>
<td>97.68</td>
</tr>
</tbody>
</table>

Batch fermentations of immobilized cells were performed in 250-ml flasks with a 175-ml working volume at a static state of 30°C for 336 h. Data are presented as means of three replicateions ± standard deviations.

**Fermentation by immobilized cells of *P. acidipropionici* TISTR 442 in 2-l fermentors supplemented with 5 N KOH and without KOH**

The fermentation of immobilized cells of *P. acidipropionici* TISTR 442 in 2-l fermentors, added with 1% CaCO₃ but without KOH, gave 15.1 ± 0.08 g/l propionic acid and 98.80% total sugar consumption at 336 h while that in 2-l fermentors, added with 1% CaCO₃ and 5 N KOH, gave 15.85 ± 0.25 g/l propionic acid and 98.54% total sugar consumption at 192 h. After 192 h incubation, the propionic acid did not increase due to the lack of total sugar in the culture. Therefore, it could be said that the maximum propionic acid in the fermentors occurred at 192 h. When fermentations in the two conditions of the fermentors were completed, it was found that the fermentation in the 2-l fermentors with 1% CaCO₃ and 5 N KOH gave more propionic acid than that in the 2-l fermentors added only with 1% CaCO₃ (Figure 3).

The total sugar of the 2-l fermentors, with pH controlled by 1% CaCO₃ and 5 N KOH, decreased more than that of the 2-l fermentors with pH controlled by only 1% CaCO₃ because by using both of 1% CaCO₃ and 5 N KOH, the controlled pH was constant at 6.5 which is optimal for propionic acid production from whey lactose by immobilized cells of *P. acidipropionici* (Yang et al., 1994). Therefore, propionibacteria would continue to grow and produce propionic acid. Moreover, the constant pH would reduce the microorganism inhibition from the acid which is the product from propionibacteria.

Yang et al., (1994) studied on propionic acid from whey by immobilized cell of *P. acidipropionici* found that produced propionic acid 20 g/l in 55 h which similar to this study.
Figure 3. Comparison of propionic acid production by immobilized cells of *P. acidipropionici* TISTR 442 in 2-l fermentors with and without 5 N KOH added at 192 h: (▲) Propionic acid by immobilized cells in 2-l fermentors with KOH added, (■) Propionic acid by immobilized cells in 2-l fermentors without KOH added, (Δ) Total sugar by immobilized cells in 2-l fermentors with KOH added, (☐) Total sugar by immobilized cells in 2-l fermentors without KOH added.

Cell recycling of fermentation by immobilized cells of *P. acidipropionici* TISTR 442 in 2-l fermentors

Figure 4 shows propionic acid production in 2-l fermentors at 216 h. In the first cycle, maximum propionic acid, 15.85±0.25 g/l, was produced by immobilized cells at 192 h which was higher than that produced by free cells (15.16±0.24 g/l at 216 h). Therefore, it was shown that immobilized cells enhanced more propionic acid production than free cells.

In the second cycle, propionic acid production decreased with a value of 13.39±0.25 g/l at 216 h and the beads collapsed and no fermentation could occur in the next cycle because the beads became less resistant to the acidity of the accumulated propionic acid. In addition, K⁺ from KOH would have replaced Ca²⁺ which decreased gel stability (Klinkenberg et al., 2001). Damirel et al., (2005) studied about the citric acid production by immobilized *Aspergillus niger* A-9 and found that the citric acid production decreased when numbers of reused immobilized *A. niger* cells increased and this process could be repeated 4 days per fermentation. Suwannakham and Yang (2005) compared the fed-batch...
and free cells fermentations of glucose by *P. acidipropionici* ATCC 4875 in a fibrous-bed bioreactor (FBB) and found that the FBB culture produced 20-59% more propionate than that by free cells fermentation.

![Figure 4.](image)

**Figure 4.** Cells recycling of propionic acid production by immobilized cells of *P. acidipropionici* TISTR 442 in 2-l fermentor: (▲) Propionic acid by immobilized cells in 2-l fermentors Batch 1, (■) Propionic acid by immobilized cells in 2-l fermentors Batch 2, (Δ) Total sugar – Batch 1, (❑) Total sugar – Batch 2

It was found in the case of the comparisons between fermentations by free cells and immobilized cells in 2-l fermentor, using both 1% CaCO₃ and 5 N KOH to control the pH, that immobilized cells gave more propionic acid than free cells at the same time (Table 4) with the reason that the entrapping method by alginate protected the cells from high acidity caused by the end-products (Ates et al., 2002). In addition, immobilization protects cells from excess oxygen, therefore, the oxygen tension inside alginate beads is lower than that in the surrounding medium which is suitable for anaerobic fermentation. Moreover, immobilized cells could be reused which would reduce the cost and time in preparing fresh seed culture.
**Table 4.** Comparisons of fermentation time, propionic acid, yield, productivity and total sugar consumption in 2-l fermentors fermented by immobilized cells and free cells of *Propionibacterium acidipropionici* TISTR 442 at 192 h incubation

<table>
<thead>
<tr>
<th>2-l Fermentors</th>
<th>Fermentation time (h)</th>
<th>Propionic acid (g/l)</th>
<th>Yield (g/g)</th>
<th>Productivity (g/l h)</th>
<th>Total sugar consumption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immobilized cells Batch 1</td>
<td>192</td>
<td>15.85 ± 0.25</td>
<td>0.48 ± 0.01</td>
<td>0.083 ± 0.0008</td>
<td>98.54</td>
</tr>
<tr>
<td>Immobilized cells Batch 2</td>
<td>192</td>
<td>12.68 ± 0.25</td>
<td>0.44 ± 0.01</td>
<td>0.066 ± 0.001</td>
<td>93.11</td>
</tr>
<tr>
<td>Free cells</td>
<td>192</td>
<td>14.89 ± 0.24</td>
<td>0.50 ± 0.008</td>
<td>0.078 ± 0.0008</td>
<td>95.27</td>
</tr>
</tbody>
</table>

Batch fermentation was performed on 2-l fermentors with a 1.4-l working volume at 30°C, 100 rpm and pH 6.5. Data are presented as means of three replications ± standard deviations.

**Antifungal effects**

The effects of propionic acid in inhibiting growth of molds and yeasts are shown in Figure 5 and Figure 6, respectively. The antifungal activities of fermented propionic acid were shown by having inhibition zones with *Aspergillus niger*, *A. oryzae*, *Rhizopus* sp., *Penicillium* sp. and *Zygosaccharomyces rouxii* with the values of 2.38, 1.94, 1.58, 3.14 and 1.68 cm, respectively. These results were similar to those of the commercial propionic acid with the inhibition zones of 2.42, 1.92, 1.68, 3.28 and 1.72 cm, respectively. Table 5 shows the statistical analyses of mold inhibition zones as affected by commercial and fermented propionic acids which were not significantly different at 95% confidence level.
<table>
<thead>
<tr>
<th>Control (H₂O)</th>
<th>Fermented propionic acid (15 mg/ml)</th>
<th>Commercial propionic acid (15 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus flavus</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>Aspergillus oryzae</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>Fusarium moniliforme</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>Rhizopus sp.</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
<tr>
<td>Trichoderma sp.</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Figure 5.** Comparisons of mold inhibitions as affected by the fermented and the commercial propionic acids
Figure 6. Comparisons of yeast inhibitions as affected by the fermented and the commercial propionic acids
Table 5. Comparisons of antifungal activity by the commercial and the fermented propionic acids using T-test

<table>
<thead>
<tr>
<th>Type of fungus</th>
<th>Inhibition zone by commercial propionic acid (cm.)</th>
<th>Inhibition zone by fermented propionic acid (cm.)</th>
<th>T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>2.42</td>
<td>2.38</td>
<td>0.622ns</td>
</tr>
<tr>
<td>Aspergillus oryzae</td>
<td>1.92</td>
<td>1.94</td>
<td>0.56ns</td>
</tr>
<tr>
<td>Rhizopus sp.</td>
<td>1.68</td>
<td>1.58</td>
<td>0.392ns</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>3.28</td>
<td>3.14</td>
<td>0.31ns</td>
</tr>
<tr>
<td>Zygosaccharomyces rouxii</td>
<td>1.72</td>
<td>1.68</td>
<td>0.147ns</td>
</tr>
</tbody>
</table>

Data are presented as means of five independent inhibition zones and statistical analyses were performed by pair sample T-test ns : P-value > 0.05, means are not significantly different at 99% confidence level * : 0.01 < P-value < 0.05, means are significantly different at 95% confidence level.

Although the fermented propionic acid was able to inhibit the growth of molds and yeasts similar to the commercial propionic acid at the same concentration, the fermented propionic acid is better than the commercial propionic acid because harmful chemicals would not occur. Lind et al., (2005) evaluated the antifungal activities of different strains of propionibacteria, e.g., Propionibacterium acidipropionici, P. freudenreichii subsp.shermanii, P. freudenreichii subsp. freudenreichii, P. thoenii and P. jensenii on sodium lactate medium at pH 7.0 and found that the growth of Rhodotorula mucilaginosa, Penicillium roqueforti and Aspergillus fumigatus was inhibited.

CONCLUSION

Whey broth supplemented with 1% CaCO$_3$ was the optimal medium in producing propionic acid by P. acidipropionici TISTR 442 as 15.16±0.24 g/l propionic acid was produced by free cells of the bacterium. The fermentation times in 2-l fermentors were faster than those in flasks. The total sugar in broth was the important factor for propionic acid production as it was shown that the remaining total sugar in broth had affected the increasing of the amount of propionic acid. For propionic acid production by immobilized cells of P. acidipropionici TISTR 442, the optimal conditions of gel formation were the controlled distances between the end of the tube to the surface of CaCl$_2$ solution which were 4-6 cm and the flow rate of the gel solution which was 7 ml/min. The fermentation by immobilized cells in 2-l fermentors with the controlled pH by 1% CaCO$_3$ and 5 N KOH produced more propionic acid than that with the controlled pH by 1% CaCO$_3$ without 5 N KOH because the constant pH in the culture obtained by the 5N KOH reduced the acidity produced by the propionibacteria. However, K$^+$ from KOH replaced Ca$^{2+}$ causing the gel stability to decrease. In this experiment, the consistent potential of immobilized cells could be maintained for 2 cycles of
fermentations and 15.85±0.25 g/l and 13.39±0.25 g/l propionic acids were produced on the first and second cycle, respectively, and these values were higher than those of the free cells (15.16±0.24 g/l) in 2-l fermentors at 216 h of the fermentation time. The fermented propionic acid as well as the commercial propionic acid from chemical processes were able to inhibit the growth of the fungi.

ACKNOWLEDGEMENTS

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Reduction in Numbers of Bacteria after Pre-milking Teat Dipping in Milking Dairy Cows

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ABSTRACT

This study was conducted to evaluate the amount of bacteria at the teat tips after pre-milking teat dipping, compared to cleaning with chlorine solution disinfectant only. Twenty-four clinically-healthy milking cows from a smallholder dairy farm in a Mae-on cooperative, Chiang Mai, Thailand, were used. After washing, udders were soaked in clean water containing 100 ppm of chlorine and dried with a single-used cloth. Then, samples from the teat tip area were collected after the teats became dry to form a control group (CONTROL). After the same preparation, a teat disinfectant was applied, as for pre-milking teat dipping, to form a treatment group (TREATMENT). Numbers of the colony-forming unit (CFU) of bacteria were counted on a standard agar plate, with those of coliform bacteria counted from a specific culture. Mean log of the CFU was compared between CONTROL and TREATMENT by the Student's T-test. A P-value <0.05 was considered as significant. Based on values of the geometric mean of both groups, total bacterial count of CONTROL was about 3.5 times higher than that of TREATMENT. A result from statistical analysis showed that total bacterial counts in CONTROL were significantly higher than those in TREATMENT. In conclusion, pre-milking teat dipping is advantageous for smallholder dairy farms in which cows are intensively cleaned with chlorine solution disinfectant.

Key words: Bacterial count, Chlorine solution disinfectant, Dairy cow, Pre-milking teat dipping
INTRODUCTION

Of farms in Thailand, smallholder dairy farms are in the majority where most dairy farmers have been trained and advised on dairy farm management, including the mastitis control program, by the Dairy Promotion Organization, Thailand. Bovine mastitis is the single most common disease syndrome in adult dairy cows, accounting for about 38% of morbidity (Smith, 1996). Mastitis is also associated with the number of zoonotic diseases in which milk acts as a vehicle of infection (Jenkins, 1982). To prevent mastitis, farmers must provide optimal milking procedures applied with the most hygienic method to minimize pathogens invading udders.

More than 95% of smallholder dairy farms in Thailand have less than 25 milking cows each, and so farmers can spend more time with their cows while milking. Approximately one hour before milking, all milking cows are washed to remove dirt from the udders, and they subsequently stand with an overhead lock until milking. Clean water with chlorine is used for teat disinfection and the udders are dried with a single-used cloth or paper towel before attaching a milking unit. In addition, post-milking dipping is accepted as a general protocol for many dairy farmers in Thailand. However, mastitis prevalence in this area remains high, especially mastitis from environmental pathogens (Boonyayatra et al., 2007). In Chiang Mai, Thailand, Boonyayatra and Chaisri (2004) studied on smallholder dairy farms and found that the prevalence of monthly subclinical mastitis ranged from 36.4% to 83.3%.

Pre-milking teat dipping, followed by post-milking teat dipping, has been established as a more effective procedure against major mastitis pathogens than post-milking teat dipping alone (Oliver et al., 1993). It remains doubtful, however, whether pre-milking teat dipping is advantageous for smallholder dairy farms in which cows are intensively cleaned with chlorine solution disinfectant. Therefore, this study evaluated the amount of bacteria at the teat tips after pre-milking teat dipping, and compared it to cleaning with only chlorine solution disinfectant.

MATERIALS AND METHODS

Animals and study design

Twenty-four clinically-healthy milking cows from a smallholder dairy farm in Mae-on cooperative, Chiang Mai, Thailand, were used. All milking cows (n = 24) were fed post-harvest corn stem and rice straw ad libitum, and concentrates according to their milk production. A majority of the cows were crossbred Holstein-Friesian, and their average overall dry matter intake was 14.5 kg/cow.

Systematic random sampling was used to assign the cows into either a control (n=12) or treatment (n=12) group. After washing, their udders were soaked in clean water with 100 ppm of chlorine and dried with a single-used cloth. Samples from the teat tip area were collected for the control group (CONTROL) once the teats became dry. After the same preparation, a teat disinfectant containing fatty and lactic acid as main ingredients (Lauricare, 3M, USA) was applied to the
treatment group (TREATMENT), as in pre-milking teat dipping. At least three-fourths of each teat was covered with pre-dip solution. Pre-dip remained in contact with the teat for 30 seconds before drying, and then swab samples were collected from the right-rear quarter of each cow. Sterile cotton buds were used for swabbing in a 1x1 cm² area of teat tips and collected in transport media. Samples were immediately transported to the laboratory of the Faculty of Veterinary Medicine, Chiang Mai University.

Laboratory procedure and statistical analysis

Numbers of the colony-forming unit (CFU) of bacteria were counted on a standard agar plate, with those of coliform bacteria counted from a specific culture. All samples were tested in a single microbiology lab by using identical bacterial growth media provided by the same manufacturer. A microbiologist made final reports. Data were entered and analyzed in Statistix 8.0. Since data were not distributed normally, a logarithm was used to transform CFU data for normal distribution. The mean log of the CFU was compared between CONTROL and TREATMENT by the Student’s T-test. A P-value <0.05 was considered as significant.

RESULTS

From the total of 12 samples from either CONTROL or TREATMENT, 11 samples (91.7%) of both groups were free from coliform bacteria which were, therefore, not used for a statistical tests for a difference of means. Data on total bacterial counts in both groups are shown in Table 1. Medians of bacterial counts, based on a 1x1 cm² area of teat tips, were 1,489 cfu and 499.5 cfu in CONTROL and TREATMENT, respectively. Based on values of the geometric mean of both groups, total bacterial count of CONTROL was about 3.5 times higher than that of TREATMENT. A result from statistical analysis showed that total bacterial counts in CONTROL were higher than those in TREATMENT (P<0.05).

Table 1. Comparison of total bacterial counts between CONTROL (conventional preparation of teats with 100 ppm chorine in smallholder dairy farms in Thailand) and TREATMENT (conventional preparation, as in CONTROL, followed by pre-milking teat dipping for 30 seconds and dried with a single-used cloth)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Min.</th>
<th>Median</th>
<th>Max.</th>
<th>Geo.Mean</th>
<th>Lower than 95% CI</th>
<th>Above 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>225</td>
<td>1,489</td>
<td>28,800</td>
<td>1,846</td>
<td>836</td>
<td>3,587</td>
</tr>
<tr>
<td>Treatment</td>
<td>12</td>
<td>90</td>
<td>499.5</td>
<td>3,330</td>
<td>518.3</td>
<td>321.5</td>
<td>949.8</td>
</tr>
</tbody>
</table>

T statistic = 2.65, Degree of freedom = 22, P = 0.01
DISCUSSION

In this study, intensive cleaning was carried out in a smallholder dairy farm by washing, soaking with chorine solution and drying to minimize the number of bacteria and coliform count at the teat tip area. Most coliform-free teats observed in this study were similar to total-coliform teats found after wetting only, followed by manual drying (Fairchild et al., 1982). Geomean numbers of bacteria in CONTROL were at the same levels as those in milk after teat preparation by either using wet towel, sanitizer and drying or disinfectant dipping and drying (Galton et al., 1984). However, the most pathogens causing mastitis around the world including Thailand were gram positive bacteria, such as environmental Streptococci and Staphylococcus spp. counting for more than 90% of total (Boonyayatra et al., 2007; Suriyasathaporn, 2010; Radostits et al., 2007). Therefore, the use of total bacteria count was a better indicator for measurement of teat dipping efficacy.

The cow-prevalence of subclinical mastitis in tropical countries ranges from 40% to 90%, including approximately 45% in India (Roman et al., 2000; Joshi and Gokhale, 2006), 38.2% in Ethiopia (Workineh et al., 2002) and from 75.9% (Karimuribo et al., 2006) to 90.3% (Kivaria et al., 2004) in Tanzania. In northern Thailand, environmental mastitis has the highest prevalence in the country, even on farms with intensive milking preparation (Boonyayatra et al., 2007). Most cows are fixed with an overhead lock but free movement of the hind quarter allows contamination with feces in the bedding area. The optimal temperature and humidity in Thailand, as a tropical country, exacerbates the numbers of environmental bacteria on the floor and also various cow surface areas, especially the udder. In this study, we showed that bacteria were effectively reduced by approximately 3.5 times after adding pre-milking teat dipping, and drying after washing and soaking with a sanitizer (Table 1). This reduction level would help lactating cows decrease intramammary infection. In western countries, it has been established that pre-dipping teats with a germicidal teat dip reduces new cases of environmental mastitis during lactation (Hogan and Smith, 1987).

The result from this study showed that the pre-dipping teat procedure was advantageous for smallholder dairy farms in which the udder was intensively washed and soaked with chorine solution disinfectant. The use of paper or cotton towels to wipe out pre-milking teat disinfectant from the skin was necessary in the case of cleaning with iodine (Galton et al., 1984; Rasmussen et al., 1991). The suggestion that iodine residues in milk originate from contaminated teat surface rather than absorption through the skin is rather valid, because iodine is a residue in milk.

In conclusion, pre-milking teat dipping is advantageous for smallholder dairy farms in which cows are intensively cleaned with chlorine solution disinfectant. The use of pre-milking teat dipping would help smallholder dairy farms reduce the prevalence of intramammary infection and mastitis.
ACKNOWLEDGEMENTS

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REFERENCES


Diazocoupling on Some $\beta$-Ketoaminato Ti$^{IV}$ Chelates

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ABSTRACT

The electrophilic substitution of p-chlorophenyldiazonium ion on $\beta$-ketoaminato Ti$^{IV}$ chelates, derived from $\beta$-dicarbonyl compounds (acetylacetone, ethylacetoacetate, benzoylacetonate) and 2-aminoethanol, with quasi-aromatic ring, was studied. The coupling products were isolated and characterized by elemental analysis, conductivity and magnetic susceptibility measurement and electronic, IR, $^1$H NMR spectral studies. The parent chelates had undergone coupling at methine carbon of the chelated ligand. The geometry around the metal ion of the product was octahedral as that of parent chelates.

Key words: Aryldiazonium ion, Metalloaromaticity, $\beta$-ketoaminato Ti$^{IV}$ chelates, Electrophilic substitution

INTRODUCTION

Metalloaromaticity is the manifestation of aromatic properties in the chelate metallocycle as introduced by Calvin and Wilson in 1945 to explain the stability of Cu(II)–1, 3-diketonate complexes (Masui, 2001). Synthesis and structures of $\beta$-diketonates were reviewed when the possibility of creating diverse metal complexes with various modes of coordination of typical chelating ligands was discussed (Skopenko et al., 2004). Acetylacetonatozinc and trifluoroacetylacetonatozinc chelates have been prepared by electrolysis and ligand exchange (Vinokurov et al., 2007). Titanium(IV) $\beta$-diketonate complexes with various $\beta$-diketones, acetylacetone, benzoylacetonate, ethylacetoacetate were synthesised by the authors (Chowdhury and Uddin, 2000).

The central hydrogen atom on metal $\beta$-diketonate chelate C,O,M ring systems can be replaced by several groups under apparently electrophilic conditions such as halogenation, thiocyanogenation, arylsulfonylation, chlorosulfonylation, nitration, acylation, formylation, chloromethylamination and dimethylaminomethylation (Collman, 1965). Electrophilic substitution of the phenyldiazonium ions at 2, 4-pentanedionates of aluminum(III), chromium(III), copper(II) and palladium(II) was reported (Krishnantutty and Micheal, 1991, 1993). Electrophilic substitution of the phenyldiazonium ions at titanium(IV) $\beta$-diketonates complexes of acetylacetone, benzoylacetonate, ethylacetoacetate was reported by authors (Chowdhury and Uddin, 2000).
Synthesis of β-ketoaminato complexes of metals, Ru(II) (Thangadurai and Natarajan, 2001), Ti(IV) (Tang et al., 2005), Co(II) (Woods et al., 2004) with Schiff bases derived from β-diketones by condensation of amines is recognised. Furthermore, likewise β-diketones metal, β-ketoamines complexes having quasi-aromatic ring systems undergo many electrophilic substitutions, typical of aromatic compounds. Trinuclear/polynuclear metal complexes resulted from Schiff base, derived by the condensation of azolo (thiazolo and triazolo)-2, 4-pentanedione and o-phenylenediamine (Mishra et al., 1993, 1997) and 2-aminoophenol and 2-aminothiophenol (Krishankutty, et al., 2007) were established. The electrophilic substitution of aryl diazonium ion on the CuII and NiII chelates of bis-(acetylacetone)ethylenediamine in aqueous medium have been studied (Sadasivan and Alaudeen, 2006). Previously, titanium(IV) complexes with dibasic tridentate ligands derived from various β-diketones, acetylacetone (acac), benzoylecetone (bzac), ethylacetoacetate (etacac) and 2-aminoethanol (AET) were reported (Chowdhury and Uddin, 1998). In continuation, electrophilic substitution of chlorobenzene diazonium ions on β-ketoaminato titanium(IV) chelates of these compounds is included in this study.

MATERIALS AND METHODS

Physical Measurements
Melting point of the ligands and complexes was determined on an electrothermal melting point apparatus. UV-absorption spectra were recorded on a Shimadzu UV-visible recording spectrophotometer (model-160). Infrared spectra were recorded on KBr pellets with Perkin-Elmer infrared spectrophotometer (Model-883). NMR spectra were recorded with JNM-PMX 60 NMR spectrophotometer using CDCl3 as solvent. Magnetic moments were determined by the Gouy method. Conductivity measurements were performed on Philips conductivity meter (model-WPA CM-25) made by WPA, Saffron Walden, England.

Elemental Analyses
Elemental analyses of some of the complexes were performed from Central Drug Research Institute (C.D.R.I), Lucknow, India. Titanium content of the diazo-coupled products was determined spectrophotometrically, following the standard procedure (Dee Snell and Biffen, 1972). All chemicals used were obtained from Aldrich Chemical Co. Ltd. Physical and analytical data for the diazo-coupled compounds are given in Table 1.
Table 1. Physical and analytical data for the diazocoupled compounds.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Colour</th>
<th>MP/°C</th>
<th>Analysis (%) (found/calcul.)</th>
<th>µ_eff (BM)</th>
<th>µ_M Ohm⁻¹ cm² mole⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti(ClBZ-Acac-AET)₂</td>
<td>Dark red</td>
<td>250</td>
<td>C-50.87 (51.24) H-5.06 (4.96)</td>
<td>8.20 (7.86)</td>
<td>dia</td>
</tr>
<tr>
<td>Ti(ClBZ-Etacac-AET)₂</td>
<td>Dark red</td>
<td>250</td>
<td>---</td>
<td>7.46 (7.18)</td>
<td>--</td>
</tr>
<tr>
<td>Ti(ClBZ-Bzac-AET)₂</td>
<td>Dark red</td>
<td>250</td>
<td>---</td>
<td>10.2</td>
<td></td>
</tr>
</tbody>
</table>

**Characteristics infra-red frequencies (cm⁻¹)**

<table>
<thead>
<tr>
<th>Comp.</th>
<th>ν(C=O)</th>
<th>ν(C=N)</th>
<th>ν(N=N)</th>
<th>ν(C-N)</th>
<th>ν(C-O)</th>
<th>ν(Ti-N)</th>
<th>ν(Ti-O)</th>
<th>Electronic spectral bands (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti(ClBZ-Acac-AET)₂</td>
<td>1620 m</td>
<td>1580 m</td>
<td>1400 m</td>
<td>1380 m</td>
<td>1240 m</td>
<td>500 m</td>
<td>415 w</td>
<td>370, 302, 270</td>
</tr>
<tr>
<td>Ti(ClBZ-Etacac-AET)₂</td>
<td>1600 s</td>
<td>1510 m</td>
<td>1490 w</td>
<td>1370 s</td>
<td>1260 s</td>
<td>540 w</td>
<td>415 s</td>
<td>370, 300, 272</td>
</tr>
<tr>
<td>Ti(ClBZ-Bzac-AET)₂</td>
<td>1640 m</td>
<td>1580 s</td>
<td>1390 s</td>
<td>1350 m</td>
<td>1270 m</td>
<td>510 w</td>
<td>410 s</td>
<td>368, 300, 273</td>
</tr>
</tbody>
</table>

s = strong, m = medium, w = weak

**Ligand Preparation**

β-dicarbonyl compounds (acetylacetone, ethylacetoacetate, benzoylacetone) (50 mmol) in ethanol (50 mL) were refluxed with 2-aminoethanol (50 mmol) for about one hr. This was cooled and allowed to stand for crystallization when crystal separated out was filtered, washed with ethanol and dried under vacuum over silica gel (Chowdhury and Uddin, 1998).

**Complex Preparation**

To a solution of the Schiff base ligand (4 mmol) in dry methanol, Ti(OPᵢ)₄ (2 mmol) was added and the mixture was refluxed for 45 min. The precipitate formed was filtered hot, washed with hot methanol and petroleum spirit (40-60°C) and dried under vacuum over calcium chloride (Chowdhury and Uddin, 1998).

**Diazonium Ion Preparation**

Benzenediazonium chloride (ClBz) was obtained through the diazotization of aniline, using sodium nitrite and hydrochloric acid. The solution of aryldiazonium salt thus obtained was immediately used for further reactions, because the salt tends to decompose slowly even at ice-bath temperature. Excess nitrous acid present in the diazonium salt solution was destroyed by adding urea (Krishnantutty and Micheal, 1991, 1993).

**Diazo-coupling Reaction**

The coupling reaction was carried out as follows. To a solution of titanium complex (2 mmol) in methanol (10 mL), kept below 5°C in an ice-salt bath, was added slowly with stirring a cold aqueous solution of the diazonium salt (4 mmol). A solution of NaOH (10⁻³ M) was used to maintain the pH of the mixture between 8 and 9. The precipitated product was filtered, washed with water, sucked dry, recrystallized from hot ethanol and dried in vacuum.
RESULTS AND DISCUSSION

The completion of the formation of aryldiazonium salt was indicated by the presence of un-reacted nitrous acid in the reaction vessel. The addition of sodium nitrite was stopped as soon as the reaction mixture just gave a blue colour with the starch potassium iodide paper. Excess nitrous acid interferes with subsequent reaction of diazonium salt produced in solution. The dry diazonium salts are unstable and explosive in nature and are seldom isolated (Bhal and Bhal, 1993). Strict care was taken for the isolation of the diazocoupled products. All the products were washed thoroughly with water to make them free from the diazonium salt. The electrophilic aryldiazonium ions were made to react with titanium chelates to yield the desired diazocoupled products. Elemental analyses of the diazo coupled products indicated that substitution had occurred on all the β-ketoaminato chelate rings of the metal complexes. Infrared spectra of the diazo-coupled products of Ti^{IV} complexes were in accord with structure shown in Figure 1.

![Diagram of reaction mechanism]

**Figure 1.** Schematic presentation for the reaction mechanism.
Infrared Spectra

The assignments were made by comparing the spectra of the complexes with those of the free ligands. The IR spectra of ligands showed strong bands at $\approx 1670 \text{ cm}^{-1}$ and $\approx 1655 \text{ cm}^{-1}$ due to $\nu(C=O)$ and $\nu(C=N)$ vibrations, respectively (Thangadurai and Natarajan, 2001, Tang et al., 2005). The $\nu(C=C)$ vibrations of the aromatic rings were observed as several medium intensity bands in the 1480–1490 cm$^{-1}$ region (Woods et al., 2004). The broad absorption bands around 3500 cm$^{-1}$ were assigned to the $\nu($OH$)$ frequency while the bands around 3265–3406 cm$^{-1}$ were assigned to the $\nu($NH$)$ frequency due to the intra-molecular hydrogen-bonded N–H proton (Thangadurai and Natarajan, 2001, Tang et al., 2005). The expected strong IR bands resulting from uncoordinated $\nu(C=O)$ and $\nu(C=N)$ vibrations shifted to a lower wave number and appeared as a new band at between 1510 and 1640 cm$^{-1}$ in the spectra of all the complexes, which showed coordination of the metal atom to the nitrogen and oxygen atoms, respectively, in the C=N and the C=O moieties. Furthermore, the intra-molecularly hydrogen–bonded O–H and N–H protons were replaced by the metal ion as confirmed by the disappearance of the broad free ligand band in the region 3200–3500 cm$^{-1}$, giving an indication of coordination of the carbonyl oxygen and amino nitrogen to the metal ion. Instead, several medium intensity bands assignable to various aliphatic and aromatic $\nu(C-H)$ vibrations appeared in the region 2851–3107 cm$^{-1}$, both in the ligands and complexes as expected. A new medium intensity band at 1390-1400 cm$^{-1}$ was assigned to $\nu($N=N$)$ vibrations (Chowdhury and Uddin, 2000). On the basis of the literature studies, bonds in the regions 1240-1270 cm$^{-1}$ and 1350-1380 cm$^{-1}$ are assigned to $\nu($C-O$)$ and $\nu($C-N$)$, respectively. The spectra of all the complexes showed additional medium intensity bands in the 410-415 cm$^{-1}$ and 500-540 cm$^{-1}$ regions, presumably due to $\nu($Ti–O$)$ and $\nu($Ti–N$)$ vibrations (Chowdhury and Uddin, 1998). Important bands which appeared in the spectra of the complexes are given in Table 1.

$^1$H-NMR Spectra

The $^1$H-NMR spectra of the diamagnetic titanium(IV) chelates also supported the formulations. Thus, the methine proton signal expected ($\delta$ 6.25 ppm) for the metal chelates was absent in the spectra of the diazocoupled compounds, a fact that it was replaced by electrophilic substitution of diazonium ions.

Electronic Spectra

The UV spectrum of the compound showed bands with maxima between 450 nm and 270 nm. In the complexes these absorption maxima shifted appreciably to lower wave numbers. Lower energy bands might be assigned to the ligand-to-metal charge-transfer and other bands were due to $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions. The complexes were coloured only through their intense charge transfer transition. The absence of bands due to d-d transition indicated the (n-1)d$'$ns$''$ electronic configuration of titanium(IV) in the complexes.
Magnetic and Conductance Measurement

The measured $\mu_{\text{eff}}$ values suggested their diamagnetic behaviour supporting 3$d^6$ electronic configuration consistent with the 4+ oxidation state and octahedral geometry of complexes. And the low molar conductance values of the complexes obtained in DMF showed them to be non-electrolytes as that of parent complexes.

CONCLUSION

The analytical, IR, $^1$H-NMR spectral data of diazocoupled compounds of titanium(IV) ketoaminato chelates suggested that coupling at methine carbon of the chelated ligand had undergone without decomposition. Together with the diamagnetic and non-electrolytic nature, 1:2 metal ligand stoichiometry of compounds as that of parent complexes conformity with Figure 1 obviously be suggested.

REFERENCES


Improvement of an Absorption Heat Transformer Performance for Upgrading Low Temperature Heat by Coupling with a Vapor Compression Heat Pump

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ABSTRACT

This paper presents a concept of a single-stage H₂O-LiBr absorption heat transformer (AHT) when it is coupled with a vapor compression heat pump (VCHP) for upgrading low temperature heat. Heat rejected at the AHT condenser is recovered by the VCHP and transferred to the AHT evaporator. For the VCHP, different types of working fluid have been considered and R-123 has been selected due to its appropriate pressure and temperature with high COP for supplying heat at around 80-90 °C.

A set of simulations has been carried out for the H₂O-LiBr AHT coupled with the R-123 VCHP to upgrade heat from hot water stream at various temperatures. With this technique, the supplied heat could be taken at the AHT generator only, not at the evaporator and the generator as the normal AHT. It could be seen that the overall COP of the combined cycle would be around 0.8, compared with 0.5 of the normal AHT. For the VCHP cycle, when it is coupled with the AHT cycle, the COP is also higher than that of a common one in upgrading the low temperature heat. For the combined cycle, lower the condensing temperature (T_c) gives a wider range of supplied hot water temperature at the generator. Higher the evaporating temperature (T_e) results in higher the absorber temperature and the overall COP.

Key words: Absorption heat transformer, Vapor compression heat pump, Simulation
INTRODUCTION

A technique to upgrade low temperature heat to a higher temperature could be taken by heat pump. The common method could be performed by a vapor compression heat pump (VCHP) of which the concept is shown in Figure 1.

Figure 1. A concept of a vapor compression heat pump for upgrading low temperature heat.

A stream of liquid at a low temperature level supplies heat to the evaporator of the VCHP, then the heat will be upgraded and generated at the condenser. This technique is commonly used to upgrade low temperature waste heat or solar heat for water heating in hotels or hospitals (Burapha and Kiatsiriroat, 2008; and Chaiyat and Kiatsiriroat, 2010).

Absorption heat transformer (AHT) is one method for upgrading low temperature heat to a higher temperature level. The schematic diagram of the AHT cycle is shown in Figure 2.
Figure 2. A schematic diagram of an absorption heat transformer (AHT).

In a conventional AHT, low temperature heat is absorbed at the AHT generator and the AHT evaporator and the heat is delivered at the AHT absorber at a higher temperature, while the AHT condenser rejects heat at a lower temperature. Theoretical and experimental studies of the AHT have been reported by various literatures. Kiatsiriroat et al., (1986) reported thermal performance of a H\textsubscript{2}O-LiBr AHT for upgrading low temperature heat such as waste heat from industrial processes or solar heat. The coefficient of performance (COP) did not exceed 0.5 because there was a high heat rejection at the AHT condenser. Xuehu et al., (2002) also reported test results of the first industrial-scale H\textsubscript{2}O-LiBr AHT in China which was used to recover waste heat released from organic vapor at 98°C in a synthetic rubber plant. The recovered heat was used to recover waste heat released from organic vapor at 98°C in a synthetic rubber plant. The AHT system was operating with a heat rate of 5,000 with a mean COP of 0.47. The payback was approximately 2 years. Sotelo and Romero (2009) presented a heat transformer absorption cycle operating with water-Carrol\textsuperscript{TM} mixture which had a higher solubility than aqueous Lithium Bromide mixture. It could be found that the coefficient of performance was higher and less crystallization risk was obtained compared with the water-Lithium Bromide solution.

It could be seen that the COP of the H\textsubscript{2}O-LiBr AHT could not be over 0.5 due to the heat rejected at the AHT condenser. If this one could be recovered and supplied back to the AHT evaporator, then the COP could be increased.
In this study, a method to improve the thermal performance of a single-stage H₂O-LiBr AHT by combining a VCHP to recover the heat rejected from the AHT condenser and supplied back at the AHT evaporator is considered. With this approach, input heat such as solar heat could supply at the AHT generator only, not at the AHT evaporator and generator. Besides, the amount of supplied heat could be less, then the number of the solar collector could be reduced. For the VCHP, an appropriate working fluid has been selected. The overall COP of the AHT coupling with the selected VCHP will be considered and compared with those of the common AHT and the common VCHP.

**MATERIALS AND METHODS**

From Figure 2, at the AHT generator, binary liquid mixture consisting of a volatile component (absorbate) and a less volatile component (absorbent) is heated at a medium temperature. Part of the absorbate boils at a low pressure (Pₐ) and a generator temperature (T₇) at state 1. The vapor condenses in the AHT condenser at a condenser temperature (T₉) to be liquid at state 2. After that, the absorbate in liquid phase is pumped to the AHT evaporator at state 3 of which the pressure (P₉) is higher than that of the AHT condenser. The AHT evaporator is heated at a medium temperature (T₈) and the absorbate in a form of vapor enters the AHT absorber which has the same pressure as the AHT evaporator at state 4. Meanwhile, liquid mixture from the AHT generator, at state 5 is pumped through a heat exchanger (state 6) into the AHT absorber to a high pressure at state 7. In the AHT absorber, the strong solution absorbs the absorbate vapor and the weak solution leaves the absorber at state 8. During absorption process, heat is released at a high temperature (T₄) which is higher than those at the AHT generator and the AHT evaporator. This liberated heat is the useful output of the AHT. The weak solution at state 8 from the AHT absorber is then throttled to a low pressure through the AHT heat exchanger at state 9 into the AHT generator again at state 10 and new cycle restarts.

The basic equations for the behavior of each component in the AHT cycle are as follows:

- **Generator**

  \[ Q_G = \dot{m}_1 h_1 + \dot{m}_5 h_5 - \dot{m}_{10} h_{10}, \]  
  \[ \dot{m}_{10} = \dot{m}_1 + \dot{m}_5, \]  
  \[ \dot{m}_{10} X_{10} = \dot{m}_5 X_5, \quad (X_1 = 0). \]  

From equations (2) and (3),

\[ \dot{m}_5 = \frac{\dot{m}_1 X_{10}}{X_5 - X_{10}}, \]  

and

\[ \dot{m}_5 = \frac{\dot{m}_1 X_{10}}{X_5 - X_{10}}. \]
• Condenser
\[ Q_C = \dot{m}_{\text{ref}} (h_1 - h_2), \quad (6) \]
\[ \dot{m}_{\text{ref}} = \dot{m}_1 = \dot{m}_2 = \dot{m}_3 = \dot{m}_4. \quad (7) \]

• Pump and solution pump
\[ W_P = (P_E - P_C) \frac{v_2 \dot{m}_2}{\eta_p}, \quad (8) \]
\[ \eta_P = (P_E - P_C) \frac{v_5 \dot{m}_5}{\eta_{SP}}, \quad (9) \]
\[ h_2 \approx h_3, \quad (10) \]
\[ h_5 \approx h_6. \quad (11) \]

• Evaporator
\[ Q_E = \dot{m}_{\text{ref}} (h_4 - h_3). \quad (12) \]

• Absorber
\[ Q_A = \dot{m}_4 h_4 + \dot{m}_7 h_7 - \dot{m}_8 h_8, \quad (13) \]
\[ \dot{m}_8 = \dot{m}_4 + \dot{m}_7, \quad (14) \]
\[ \dot{m}_8 X_8 = \dot{m}_7 X_7. \quad (15) \]

• Heat exchanger
\[ Q_{\text{HX}} = \dot{m}_8 C_P (T_8 - T_9) = \dot{m}_6 C_P (T_7 - T_6) = \varepsilon_{\text{HX}} (mC_P)_{\text{min}} (T_8 - T_6), \quad (16) \]
\[ \dot{m}_8 = \dot{m}_9, \quad (17) \]
\[ \dot{m}_6 = \dot{m}_7. \quad (18) \]

• Expansion valve
\[ h_9 = h_{10} \text{ (Throttling process)}. \quad (19) \]

• Flow ratio (FR)
\[ \text{FR} = \frac{\dot{m}_5}{\dot{m}_{\text{ref}}}. \quad (20) \]

• Gross temperature life (GTL)
\[ \text{GTL} = T_8 - T_4. \quad (21) \]

• Coefficient of performance (COP)
\[ \text{COP}_{\text{AHT}} = \frac{Q_A}{Q_E + Q_G + W_P + W_{SP}}. \quad (22) \]
Figure 3 shows a schematic diagram of an AHT coupling with a VCHP and the combined cycle is called Compression/Absorption Heat Transformer (CAHT). The heat rejected at the AHT condenser is recovered by the VCHP, then the heat is upgraded and generated back to the AHT evaporator.

The basic equations for the behavior of each component in the VCHP cycle as presented in Figure 3 are as follows:

- **Evaporator**
  \[ Q_{Er} = \dot{m}_r (h_{1r} - h_{4r}), \]  
  \[ \dot{m}_r = \dot{m}_{1r} = \dot{m}_{2r} = \dot{m}_{3r} = \dot{m}_{4r}. \]  

- **Compressor**
  \[ W_{Comp} = \dot{m}_r (h_{2r} - h_{1r}), \]  
  \[ S_{1r} = S_{2r} \text{ (Isentropic process)}, \]  
  \[ \eta_{Comp} = \frac{h'_{2r} - h_{1r}}{h_{2r} - h_{1r}}. \]  

- **Condenser**
  \[ Q_{Cr} = \dot{m}_r (h_{2r} - h_{3r}). \]  

- **Expansion valve**
  \[ h_{3r} = h_{4r} \text{ (Throttling process)}. \]  

- **Coefficient of performance (COP)**
  \[ \text{COP}_{VCHP} = \frac{Q_{Cr}}{W_{Comp}}. \]
Then, the overall coefficient of performance (COP) of the CAHT will be:

$$\text{COP}_{\text{CAHT}} = \frac{Q_A}{Q_G + W_P + W_{SP} + W_{\text{Comp}}}$$  \hspace{1cm} (31)
It could be seen that $W_{\text{Comp}}$ in equation (31) is less than $Q_E$ in equation (22), thus the COP $\text{CAHT}$ is higher than the COP $\text{AHT}$.

Table 1. Physical properties of working fluids.

<table>
<thead>
<tr>
<th>Working Fluid</th>
<th>R-22</th>
<th>R-290</th>
<th>R-134a</th>
<th>R-717</th>
<th>R-123</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formulae</td>
<td>CHClF$_2$</td>
<td>C$_3$H$_8$</td>
<td>CF$_3$CH$_2$F</td>
<td>NH$_3$</td>
<td>CHCl$_2$CF$_3$</td>
</tr>
<tr>
<td>Molecular mass (kg/kmol)</td>
<td>86.46</td>
<td>44.10</td>
<td>102.03</td>
<td>17.03</td>
<td>152.93</td>
</tr>
<tr>
<td>Critical temperature (°C)</td>
<td>96.14</td>
<td>96.68</td>
<td>101.06</td>
<td>132.25</td>
<td>183.68</td>
</tr>
<tr>
<td>Critical pressure (MPa)</td>
<td>4.99</td>
<td>4.25</td>
<td>4.06</td>
<td>11.33</td>
<td>3.66</td>
</tr>
<tr>
<td>Critical density (kg/m$^3$)</td>
<td>523.84</td>
<td>218.50</td>
<td>511.90</td>
<td>225.00</td>
<td>550.00</td>
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<tr>
<td>Boiling point (°C)</td>
<td>-40.81</td>
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<td>-26.07</td>
<td>-33.33</td>
<td>27.82</td>
</tr>
<tr>
<td>Latent heat of vaporization at 40 °C (kJ/kg)</td>
<td>164.24</td>
<td>302.30</td>
<td>160.88</td>
<td>1089.82</td>
<td>164.04</td>
</tr>
<tr>
<td>Flammability</td>
<td>NO</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
<td>NO</td>
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<tr>
<td>Toxicity</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>ALT (Year, Atmosphere Life Time)</td>
<td>13.3</td>
<td>&lt;1</td>
<td>14</td>
<td>&lt;1</td>
<td>1.4</td>
</tr>
<tr>
<td>ODP (CO$_2$-related, Ozone Depletion Potential)</td>
<td>0.034</td>
<td>~0</td>
<td>0.0015</td>
<td>~0</td>
<td>0.02</td>
</tr>
<tr>
<td>GWP (100 Years, Global Warming Potential)</td>
<td>1780</td>
<td>0</td>
<td>1320</td>
<td>0</td>
<td>76</td>
</tr>
</tbody>
</table>

Selection Working Fluid of the VCHP

Five working fluids, R-22 (Chlorodifluoromethane), R-290 (Propane), R-134a (1, 1, 1, 2-Tetrafluoroethane), R-717 (Ammonia) and R-123 (2, 2-Dichloro-1,1,1-trifluoroethane) for heat pump have been considered as working fluid in the VCHP Table 1 shows physical properties of the working fluids (NIST, 2000). The working conditions for the evaluation are:

1. The VCHP evaporator temperature ($T_{\text{Er}}$) is at 40°C.
2. Total cooling capacity ($Q_{\text{Er}}$) is 10 kW.
3. Required hot water temperature ($T_{\text{HW,o}}$) is around 80-85°C (the VCHP condenser temperature ($T_{\text{C}}$) is at 90°C)
4. No pressure drops at the VCHP condenser and the VCHP evaporator.
5. Isentropic efficiency of compressor ($\eta_{\text{Comp}}$) is 80%.
6. Degree of superheating (SH) is 5.0°C.
7. Degree of subcooling (SC) is 5.0°C.
8. The properties of working fluids are based upon REFPROP (NIST, 2000).

The indicators used to identify the appropriate working fluid are mass of refrigerant per unit heat output, volume flow rate of refrigerant, high-side pressure, refrigerant temperature at the compressor outlet, pressure ratio and heating COP. Figure 4 shows the results of the selected refrigerants.
A) Mass of refrigerant per unit heat output, (g/kJ)
B) Vapor volume flow rate, \((10^{-2} \cdot m^3/kg)\)
C) Displacement volume, \((10 \cdot m^3/h)\)
D) Discharge pressure, \((10 \cdot bar)\)
E) Discharge temperature, \((10^2 \cdot ^\circ C)\)
F) Pressure ratio, (-)
G) COP_{hp}, (-).

**Figure 4.** The results for the selected refrigerants.

Mass of refrigerant per unit heat output indicates the amount of refrigerant used in the VCHP cycle compared with the amount of heat generated. If the value is high, it means that for the same amount of generated heat, high amount of refrigerant is needed which results in a large scale of the components and high compression work. From Figure 4, it could be seen that R-290 and R-717 seem to be appropriate since the values of mass of refrigerant per unit heat output are around 3/5 and 1/8 of R-22. However, these refrigerants are flammable and R-717 is quite toxic and not compatible with copper which is a general material used in the heat pump.

The volume flow rate of refrigerant at the compressor inlet should be selected to be matched with the displacement volume of compressor. If the value is high, a big size of compressor is required. Figure 4 also shows that R-123 gives a high value which is around 600% of that from R-22 which means that the displacement volume of its compressor is 6 times of R-22 compressor. Therefore, if R-123 is taken, an open-type compressor should be used. From Figure 4, it could be seen that R-717 gives the best solution but the user should be aware of the corrosion of the refrigerant and the suitable lubricant.
Discharge pressure is the maximum pressure of refrigerant in the heat pump cycle. If the value is high, the thickness of coil and the fittings should have a special design which results in high initial investment. Moreover, high compression work is consumed. It could be seen that R-123 gives the best solution since the pressure is lowest compared with the other refrigerants. Discharge temperature of refrigerant at the compressor discharge is the maximum temperature in the heat pump cycle. If this is too high, the lubricant will not be stable. R-123, R-134a and propane are suitable for this case. The latter one is flammable, thus it is not appropriate to be used at a high temperature.

Pressure ratio is the ratio of the condenser pressure to the evaporator pressure. If this value is high, the compressor will consume high power input. From Figure 4, propane gives the lowest value while R-123 gives the highest one.

Figure 4 also shows the ideal heating COP\textsubscript{hp} of the heat pump with different types of refrigerant. From the Figure, it could be seen that the best refrigerant is R-123 which gives the highest COP.

From the above results, it could be seen that R-123 gives the suitable refrigerant in terms of energy consumption for the heat pump for generating heat at about 80-85°C due to its low maximum pressure for the heat pump compressor, and highest COP is obtained.

**Working Conditions for the CAHT Analysis**

All calculations of the CAHT are based on the systems presented in Figure 3. The water-Lithium Bromide is the working pair of the AHT and R-123 is the refrigerant of the VCHP. The working conditions for the evaluation are:

1. Supplied heat of the AHT is hot water temperature (T\textsubscript{H5,i}) at around 50-95°C.
2. Supplied hot water flow rate (\dot{m}_{HS}) is 1 liter/s.
3. Minimum concentration of weak H\textsubscript{2}O-LiBr solution (X\textsubscript{min}) is 45% LiBr.
4. Minimum concentration difference of strong and weak H\textsubscript{2}O-LiBr solution is 2% LiBr.
5. No pressure drops at the AHT condenser, the AHT generator, the AHT evaporator, the AHT absorber and the AHT heat exchanger.
6. Isentropic efficiency of water pump (\eta\textsubscript{p}) and solution pump (\eta\textsubscript{SP}) is 85%.
7. Effectiveness of the AHT heat exchanger (\epsilon\textsubscript{HX}) is 85%.
8. Temperature difference between the outlet supplied hot water and the AHT generator is 5°C.
9. Temperature difference between the outlet useful water and the AHT absorber is 5°C.
10. Temperature difference between the outlet cooling water and the AHT condenser is 5°C.
11. Temperature difference between the outlet supplied hot water and the AHT evaporator is 5°C.
12. The properties of H\textsubscript{2}O-LiBr solution are shown in Appendix.

Figure 5 shows the steps for calculating the CAHT performance.
Figure 5. Flow chart of the simulation program for evaluating the CAHT performance.
RESULTS AND DISCUSSION

**Effect of the AHT condenser temperature on the CAHT performance**

Figure 6 shows the absorber temperature \( T_A \) with various values of the supplied hot water temperature at the generator of the normal AHT cycle and the CAHT cycle. It could be seen that \( T_A \) of the CAHT is nearly constant at around 100°C because the AHT evaporator temperature \( T_E \) is constant (about 80°C) while that of the normal AHT varies with the hot water temperature. It could also be seen that, for the CAHT, the condensing temperature \( T_C \) affects \( T_A \) only slightly. For lower, \( T_C \), the CAHT cycle could operate with a wide range of hot water temperature.

Figure 7 shows the variations of the overall COP of the CAHT cycle at various hot water temperatures. The overall COP of the CAHT cycle is nearly constant with various supplied hot water temperatures at the generator. Compared to the COP of the AHT cycle, the CAHT performance is around 1.6 times of the AHT value (COP of the AHT \( \approx 0.5 \)). Higher the condensing temperature, \( T_C \), results in higher the COP of the CAHT due to the less power at the VCHP compressor.

**Effect of the AHT evaporator temperature on the CAHT performance**

Figure 8 shows the variation of the CAHT absorber temperature \( T_A \) with the evaporator temperature \( T_E \). It could be seen that \( T_A \) increases significantly with \( T_E \). Higher \( T_E \) will give higher pressure in the absorber which results in higher \( T_A \) value.

Similarly, the overall COP of the CAHT cycle could be improved by decreasing \( T_E \). Lower \( T_E \) requires less VCHP compression work \( W_{\text{Comp}} \) which results in higher COP of the CAHT cycle. The results are shown in Figure 9.
**Figure 6.** Effect of the supplied hot water temperature on the absorber temperature for the normal AHT and the CAHT.

**Figure 7.** The overall COP on $T_C$ of the normal AHT and the CAHT.
Figure 8. The effect of the AHT $T_E$ on the AHT $T_A$ of the CAHT.

Figure 9. The overall COP on $T_E$ of the normal AHT and the CAHT.
CONCLUSION

From this study, the conclusions are as follows:

1. The suitable working fluid of the VCHP is R-123 due to its low operating pressure and high COP of the VCHP cycle for supplying heat at around 80-90°C.

2. The CAHT can produce upgraded heat at a nearly constant temperature at the absorber and the overall COP. Lower the condensing temperature ($T_C$) gives a wider range of supplied hot water temperature at the generator. Higher the evaporating temperature ($T_E$) results in high absorber temperature and the overall COP.

3. The overall COP of the CAHT cycle can increase around 80% over that of the normal AHT (the overall COP of the AHT around 0.5).

ACKNOWLEDGEMENTS

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REFERENCES


APPENDIX

A.1 Enthalpy-Concentration and Temperature for lithium bromide-water solutions (ASHRAE, 2001)

For Concentration $x < 40\%$LiBr

Solution temperature range $15 < t < 165^\circ$C

$$h = 21.4817157 - 2.38366711X + 3.90458186t + 0.03625001X^2$$
$$+ 5.25010607 \times 10^{-4}t^2 - 0.0369249939tX, \text{ kJ/kg}$$

For Concentration $40 \leq x < 70\%$LiBr

Solution temperature range $15 < t < 165^\circ$C

$$h = \sum_{n=0}^{4} A_n X^n + t \sum_{n=0}^{4} B_n X^n + t^2 \sum_{n=0}^{4} C_n X^n, \text{ kJ/kg}$$

$A_0 = -2024.33$ $B_0 = 18.2829$ $C_0 = -3.7008214 \times 10^{-2}$

$A_1 = 163.309$ $B_1 = -1.1691757$ $C_1 = 2.8877666 \times 10^{-3}$

$A_2 = -4.88161$ $B_2 = 3.248041 \times 10^{-3}$ $C_2 = -8.1313015 \times 10^{-4}$

$A_3 = 6.302948 \times 10^{-2}$ $B_3 = -4.034184 \times 10^{-4}$ $C_3 = 9.9116628 \times 10^{-5}$

$A_4 = -2.913705 \times 10^{-4}$ $B_4 = 1.8520569 \times 10^{-5}$ $C_4 = -4.4441207 \times 10^{-6}$

A.2 Solution Temperature-Refrigerant Temperature and Saturation pressure (ASHRAE, 2001)

For Refrigerant $-15 < t' < 110^\circ$C

Solution temperature $5 < t < 175^\circ$C

Concentration $45 < X < 70\%$LiBr

$$t = \sum_{n=0}^{3} B_n X^n + t \sum_{n=0}^{3} A_n X^n, ^\circ C$$

$$t' = (t - \sum_{n=0}^{3} B_n X^n) / \sum_{n=0}^{3} A_n X^n, ^\circ C$$

$$\log P = C + D/T' + E/T'^2, \text{ kPa}; T' = K$$

$$T' = \frac{-2E}{D + [D^2 - 4E(C - \log P)]^{0.5}}$$

$A_0 = -2.00755$ $B_0 = 124.937$ $C = 7.05$

$A_1 = 0.16976$ $B_1 = -7.71649$ $D = -1596.49$

$A_2 = -3.133362 \times 10^{-3}$ $B_2 = 0.152286$ $E = -104095.5$

$A_3 = 1.97668 \times 10^{-5}$ $B_3 = -7.9509 \times 10^{-4}$
A.3 Equilibrium Chart for Aqueous Lithium Bromide Solutions (ASHRAE, 2001)

For Solution temperature \( t < 250^\circ C \)
Concentration \( 30 < X < 65\% \text{LiBr} \)
\[
\bar{n}(t,m) = \bar{n}_o(t)[1+d_0(t)m+d_1(t)m^{1.5}+d_2(t)m^2], \text{kg/m}^3
\]
\[
m = \frac{w}{M_o}(1-w), \text{mole/kg}
\]
\[
d_j(t) = \sum_{i=0}^{4} C_{ji}t^i
\]
\[
\bar{n}_o(t) = \text{Density of pure water, kg/m}^3
\]
\[
M_o = 0.086845 \text{ kg/mole}
\]

Table of Coefficients \( C_{ji} \)

<table>
<thead>
<tr>
<th>j/i</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.9979 E-2</td>
<td>-9.36591 E-5</td>
<td>1.1770035 E-6</td>
<td>-2.829722 E-9</td>
<td>7.963374 E-12</td>
</tr>
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<td>1</td>
<td>-7.30855 E-3</td>
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<td>-3.458841 E-8</td>
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<td>1.085224 E-12</td>
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<td>2</td>
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<td>-1.565022 E-8</td>
<td>2.082693 E-10</td>
<td>-3.761121 E-13</td>
</tr>
</tbody>
</table>
A.5 Heat capacity of lithium bromide-water solutions (Kaita, 2000)

For Solution temperature 40 < t < 210˚C

Concentration 40 < X < 65%LiBr

\[ C_p = (A_0 + A_1X) + (B_0 + B_1X)t, \text{ kJ/kg}^{-\circ}\text{C} \]

\[ A_0 = 3.462023 \quad B_0 = 1.3499 \times 10^{-3} \]
\[ A_1 = -2.679895 \times 10^{-2} \quad B_1 = -6.55 \times 10^{-6} \]

Nomenclature

A  Area, 
Cp  Heat capacity, (kJ/kg·K) 
COP  Coefficient of performance, (-) 
h  Enthalpy, (kJ/kg) 
LMTD  Log mean temperature difference, (-) 
m  Mass flow rate, (kg/s) 
P  Pressure, (bar) 
Q  Heat rate, (kW) 
R  Refrigerant, (-) 
v  Specific volume, (m³/kg) 
s  Entropy, (kJ/kg·K) 
SC  Subcooling, (˚C) 
SH  Superheating, (˚C) 
T  Temperature, (˚C) 
U  Overall heat transfer coefficient, (W/m²·K) 
W  Work, (kW) 
X  Concentrate, (%LiBr)

Greek symbol

\( \psi \)  Efficiency, (%) 
\( \eta \)  Effectiveness, (%) 
\( \rho \)  Density, (kg/m³)

Subscript

A  Absorber 
act  Actual 
bulk  Bulk temperature 
C  Condenser 
Comp  Compressor 
CW  Cooling water 
e  Super heat 
E  Evaporator 
H  High 
HS  Heat source 
HW  Hot water
HX  Heat exchanger
i    Inlet
L    Low
max  Maximum
min  Minimum
o    Outlet
P    Pump
r    Compression cycle
ref  Refrigerant
S    Start
SP   Solution pump
UF   Useful
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