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Chemical Transformation in the Atmosphere

Once released in the atmosphere, chemical species are transported and gradually transformed by chemical and physical processes. A major transformation path is provided by oxidation processes of reduced species emitted at the surface, and the formation of secondary compounds. Oxidation in the atmosphere take place through reaction primarily with the hydroxyl radical (OH) during daytime. Reactions with ozone (O₃) and with the nitrate radical (NO₃) are other possible pathways, but in most cases, are only significant during nighttime. In the troposphere the OH radical is produced by oxidation of water vapour by the electronically excited oxygen atom, itself produced by photolysis of ozone at wavelengths shorter than 320 nm. Once photochemically produced, OH is rapidly converted to the hydroperoxy radical (HO₂) by reaction with ozone, carbon monoxide, methane and other hydrocarbons. HO₂ is converted back to OH by reaction with ozone and nitric oxide (NO). The reaction of HO₂ with NO produces NO₂, which is photolized to nitric oxide (NO) and atomic oxygen (O). It leads to a net production of ozone since the atomic O reacts rapidly with O₂ to form O₃. At high concentrations of nitrogen oxides, OH is converted to nitric acid (HNO₃). This latter mechanism leads to a net loss of hydroxyl radicals when HNO₃, which is very soluble in water, is removed from the atmosphere by wet scavenging rather than being photolyzed to form OH and NO₂ (or H and NO₃). Other loss processes for odd hydrogen radicals (HOₓ=OH+HO₂) include the reaction of OH with HO₂, which produces water vapour (H₂O), and of HO₂ with itself, which produces hydrogen peroxide (H₂O₂), another highly soluble compounds.

Compositional Changes of the Uterine Arteries in Japanese and Thai with Aging

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ABSTRACT

To elucidate compositional changes of the uterine artery with aging, the authors investigated age-related changes of elements in the uterine arteries of Japanese and Thai by direct chemical analysis. After ordinary dissections at Nara Medical University and Chiang Mai University were finished, the uterine arteries were resected from the subjects. After ashing of arteries with nitric acid and perchloric acid, element contents were determined by inductively coupled plasma-atomic emission spectrometry. It was found that a higher accumulation of Ca occurred in the uterine artery with aging in comparison with other three branches of the internal iliac artery. In the uterine arteries of both Japanese and Thai, the Ca, P and Na content increased significantly with aging. In the uterine artery of Thai, the Ca content began to increase in the forties and increased up to the seventies. As far as the uterine arteries in the subjects more than 60 years of age, the extent of Ca accumulation in the uterine arteries of Thai was one half of that in the uterine arteries of Japanese. It should be noted that the Ca accumulation occurred in the uterine artery independently of other arteries, such as the thoracic and abdominal aortas and the coronary, common carotid, splenic and common iliac arteries.

Key words: Uterine artery, Internal iliac artery, Calcium, Phosphorus, Atherosclerosis, Aging

INTRODUCTION

There are several reports (Camiel et al., 1967; Fisher and Hamm, 1975; Kadziołka et al., 1985; Punnonen et al., 1995; Crawford et al., 1997) on calcification or atherosclerosis of the uterine artery. Histological and pathologic studies
(Crawford et al., 1997) revealed that atherosclerosis of the uterine artery occurred more frequently in postmenopausal women than in premenopausal women. However, few studies had been conducted on the element contents in the uterine artery by direct chemical analysis. Therefore, the authors investigated first whether the extent of Ca accumulation was different between the branches of the internal iliac arteries. Next, the authors focused on the uterine artery and investigated age-related changes of elements in the uterine arteries of both Japanese and Thai. It was found that a higher accumulation of Ca occurred in the uterine artery in comparison with other branches of the internal iliac artery and that there was a significant difference in age-related changes of the Ca content between the uterine arteries of Japanese and Thai.

MATERIALS AND METHODS

Sampling of Arteries

Japanese cadavers were treated by injection of a mixture of 36% ethanol, 13% glycerin, 6% phenol, and 6% formalin through the femoral artery (Tohno, Y. et al, 1985). Thai cadavers were treated by injection of a mixture of 26% methanol, 14% glycerin, 3% phenol, 14% formalin, 0.34 M potassium nitrate, and 14 mM arsenic oxide through the femoral artery (Tohno, Y. et al., 2001a). After ordinary dissections by medical students at Nara Medical University and Chiang Mai University were finished, the uterine arteries were resected from the subjects. The distal sites of the uterine arteries were used in the present study.

Determination of Elements

The samples of arteries were washed thoroughly with distilled water and were dried at 80°C for 16 h. After 1 mL conc. nitric acid was added to the dry samples, the mixtures were heated at 100°C for 2 h. After the addition of 0.5 mL conc. perchloric acid, they were heated at 100°C for an additional 2 h. The samples were adjusted to a volume of 10 mL by adding ultrapure water and were filtered through filter paper (No. 7; Toyo Roshi, Osaka, Japan). The resulting filtrates were analyzed with an inductively coupled plasma-atomic emission spectrometer (ICPS-7510; Shimadzu, Kyoto, Japan) (Tohno, Y. et al., 1996). The conditions were 1.2 kW of power from a radio-frequency generator, a plasma argon flow rate of 1.2 L/min, a cooling gas flow of 14 L/min, a carrier gas flow of 1.0 L/min, an entrance slit of 20 μm, an exit slit of 30 μm, a height of observation of 15 mm, and an integration time lapse of 5 s. The element amount was expressed on a dry-weight basis.

Statistical Analysis

Statistical analyses were performed using the GraphPad Prism version 3.0 (GraphPad Software Inc., San Diego, CA, USA). Pearson’s correlation was used to investigate the association between parameters. A two-tailed unpaired Student’s t test was used to compare differences between groups. A p-value of less than 0.05 was considered to be statistically significant. Data were expressed as the
RESULTS

Ca Content in Four Branches of the Internal Iliac Arteries

To examine whether the extent of Ca accumulation with aging was different between the branches of the internal iliac artery, the authors investigated the Ca content of four branches of the internal iliac artery, such as the uterine, internal pudendal, umbilical, and obturator arteries in ten Japanese women subjects. The Japanese women subjects ranged in age from 52 to 96 years (average age=77.4±13.1 years). Table 1 indicates the average content of Ca in the four branches of the internal iliac artery. The average content of Ca was highest in the uterine arteries and decreased in order of the internal pudendal, umbilical and obturator arteries. A significant difference in the average content of Ca was found between the uterine and either umbilical or obturator arteries, but it was not found between the uterine and internal pudendal arteries. The average content of Ca in the uterine arteries corresponded to 46-fold the amount of that in the obturator arteries. This result indicated clearly that the extent of Ca accumulation was different among the four branches of the internal iliac artery at old age.

Table 1. Comparison of the Average Content of Ca in the Branches of the Internal Iliac Arteries.

<table>
<thead>
<tr>
<th>Artery</th>
<th>Average Content of Ca (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine</td>
<td>68.74±84.81</td>
</tr>
<tr>
<td>Internal Pudendal</td>
<td>26.02±56.69</td>
</tr>
<tr>
<td>Umbilical</td>
<td>3.40±2.29*</td>
</tr>
<tr>
<td>Obturator</td>
<td>1.48±2.16*</td>
</tr>
</tbody>
</table>

Note: *A p value between the uterine and either umbilical or obturator arteries was < 0.05.

Age-Related Changes of Elements in the Uterine Arteries of Japanese and Thai

To elucidate compositional changes of the uterine artery with aging, the authors investigated age-related changes of elements in the uterine arteries of 27 Japanese and 28 Thai women subjects. Japanese women subjects ranged in age from 58 to 99 years (average age=82.7±10.1 years). Thai women subjects ranged in age from 27 to 86 years (average age=63.3±17.7 years).

Figure 1 shows age-related changes of the Ca, P and Na contents in the uterine arteries of both Japanese and Thai. In the uterine arteries of Japanese, the correlation coefficients between age and element contents were estimated to be 0.430 (p=0.025) for Ca, 0.425 (p=0.027) for P and 0.526 (p=0.005) for Na. Significant direct correlations were found between age and either Ca or P content and a very significant direct correlation was found between age and Na content in
the uterine arteries of Japanese. However, no significant correlations were found between age and element contents, such as Mg, Zn and Fe in the uterine arteries of Japanese.

![Figure 1.](image)

**Figure 1.** Age-related changes of the Ca (a), P (c) and Na (e) contents in the uterine arteries of Japanese and of the Ca (b), P (d) and Na (f) contents in the uterine arteries of Thai.

In the uterine arteries of Thai, the correlation coefficients between age and element contents were estimated to be 0.425 ($p=0.024$) for Ca, 0.419 ($p=0.026$) for P and 0.383 ($p=0.045$) for Na. Significant direct correlations were found between age and element contents, such as Ca, P and Na in the uterine arteries of Thai. However, no significant correlations were found between age and element contents, such as Mg, Zn and Fe in the uterine arteries of Thai. The common finding that there were significant direct correlations between age and element contents, such as Ca, P and Na was obtained in the uterine arteries of both Japanese and Thai.

Figure 2 shows age-related changes of the Ca content in the uterine arteries of both Japanese and Thai. The linear slopes drawn with the computer software were different between the uterine arteries of Japanese and Thai. The difference between the two slopes was significant, because a $p$ value was 0.046.
Comparison in the Average Content of Elements Between the Uterine Arteries of Japanese and Thai

Figure 3 shows the average content of Ca in the uterine arteries of Japanese and Thai by age group. In the uterine arteries of Japanese, the average content of Ca was significantly high in the seventies and increased remarkably in the eighties. The average content of Ca in the eighties corresponded to 2.7-fold the amount of that in the seventies. In the uterine arteries of Thai, the average content of Ca was significantly high in the sixties and increased remarkably in the seventies. The average content of Ca in the seventies corresponded to 6-fold the amount of that in the forties.

Figure 2. Age-related changes of the Ca content in the uterine arteries of both Japanese (open circle) and Thai (solid circle). The equation of Japanese, y=3.328x-193.4; the equation of Thai, y=0.805x-27.7. The difference between the two slopes in Fig. 2 was significant, because a p value was 0.046.

Figure 3. Comparison in the average content of Ca in the uterine arteries of Japanese and Thai by age group. The open and crossed bars indicate Japanese and Thai, respectively.
In comparison with the uterine arteries of Thai, the average content of Ca in the uterine arteries of Japanese was similar to in the seventies, but it was two times higher in the eighties. However, the difference between their average contents of Ca in the eighties was not statistically significant.

Table 2 indicates the incidence of the uterine arteries of Japanese and Thai with the Ca content more than 10 mg/g which is not contained in a normal artery. In Japanese, the incidence of the uterine artery with the high Ca content was 100% in the seventies, 83% in the eighties and 100% in the nineties. In Thai, the incidence of the uterine artery with the high Ca content was 57% in the sixties, 100% in the seventies and 50% in the eighties. It is interesting that in Thai, the incidence of the uterine artery with the high Ca content decreased from 100% in the seventies to 50% in the eighties.

As far as the subjects more than 60 years of age are concerned, the incidence of the uterine artery with the high Ca content was 92% in Japanese and 68% in Thai. The incidence of the uterine artery with the Ca content more than 10 mg/g was higher in Japanese than in Thai. Furthermore, as far as the subjects more than 60 years of age are concerned, the average content of Ca in the uterine arteries was 82.73±79.80 mg/g in Japanese and 31.57±39.32 mg/g in Thai. In the uterine arteries more than 60 years of age, both the incidence of the uterine artery with the Ca content more than 10 mg/g and the average content of Ca were higher in the Japanese than in the Thai.

### Table 2. Incidence of the Uterine Arteries of Japanese and Thai with the Ca Content more than 10 mg/g.

<table>
<thead>
<tr>
<th>Age Group (Years)</th>
<th>Incidence (%)</th>
<th>Japanese</th>
<th>Thai</th>
</tr>
</thead>
<tbody>
<tr>
<td>30s</td>
<td>NA</td>
<td>50% (1/2)</td>
<td></td>
</tr>
<tr>
<td>40s</td>
<td>NA</td>
<td>20% (1/5)</td>
<td></td>
</tr>
<tr>
<td>50s</td>
<td>100% (1/1)</td>
<td>0 % (0/1)</td>
<td></td>
</tr>
<tr>
<td>60s</td>
<td>100% (2/2)</td>
<td>57% (4/7)</td>
<td></td>
</tr>
<tr>
<td>70s</td>
<td>100% (6/6)</td>
<td>100% (6/6)</td>
<td></td>
</tr>
<tr>
<td>80s</td>
<td>83% (10/12)</td>
<td>50% (3/6)</td>
<td></td>
</tr>
<tr>
<td>90s</td>
<td>100% (6/6)</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

Note: The number of cases is indicated in parentheses. NA indicates that the specimen was not analyzed.

### Relationships Among Elements in the Uterine Arteries of Japanese and Thai

The relationships among element contents were examined in the uterine arteries of both Japanese and Thai. In the uterine arteries of Japanese, the correlation coefficients were estimated to be 0.922 \((p<0.0001)\) between Ca and P contents, 0.860 \((p<0.0001)\) between Ca and Mg contents and 0.973 \((p<0.0001)\) between P and Mg contents (Table 3). In the uterine arteries of Thai, the correlation...
coefficients were estimated to be 0.986 (p<0.0001) between Ca and P contents, 0.959 (p<0.0001) between Ca and Mg contents and 0.946 (p<0.0001) between P and Mg contents (Table 3). Extremely significant direct correlations were found between Ca and P contents, between Ca and Mg contents and between P and Mg contents in the uterine arteries of both Japanese and Thai. As shown in Table 3, extremely significant direct correlations were also found between Zn and element contents, such as Ca, P and Mg, and between Na and element contents, such as Ca, P, Mg and Zn. However, no significant correlations were found regarding Fe, except for a significant direct correlation between Zn and Fe contents. Therefore, extremely significant direct correlations were found among the contents of Ca, P, Mg, Zn and Na in the uterine arteries of both Japanese and Thai. This meant that as Ca increased in the uterine artery, P, Mg, Zn and Na also increased in the artery.

**Table 3.** Relationships Among Element Contents in the Uterine Arteries of Japanese and Thai.

<table>
<thead>
<tr>
<th>Element</th>
<th>P</th>
<th>Mg</th>
<th>Zn</th>
<th>Fe</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>0.922</td>
<td>0.860</td>
<td>0.805</td>
<td>0.125</td>
<td>0.804</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(0.536)</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>0.986</td>
<td>0.959</td>
<td>0.657</td>
<td>0.209</td>
<td>0.967</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(0.286)</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.973</td>
<td>0.793</td>
<td>-0.014</td>
<td>0.773</td>
<td>0.982</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(0.945)</td>
<td>(&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.946</td>
<td>0.667</td>
<td>0.235</td>
<td>0.921</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(0.0001)</td>
<td>(0.228)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>0.778</td>
<td>0.759</td>
<td>-0.048</td>
<td>0.731</td>
<td></td>
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<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(0.813)</td>
<td>(&lt;0.0001)</td>
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<tr>
<td></td>
<td>0.759</td>
<td></td>
<td>0.277</td>
<td>0.921</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(0.154)</td>
<td>(0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>0.388</td>
<td></td>
<td>0.388</td>
<td>0.647</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.046)</td>
<td></td>
<td>(0.004)</td>
<td>(0.0003)</td>
<td></td>
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<tr>
<td></td>
<td>0.526</td>
<td></td>
<td></td>
<td>0.651</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.004)</td>
<td></td>
<td></td>
<td>(0.0002)</td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td></td>
<td></td>
<td></td>
<td>0.115</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.569)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.233</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.233)</td>
<td></td>
</tr>
</tbody>
</table>

Note: The upper roman and lower italic numerals indicate Japanese and Thai, respectively. p-Values are indicated in parentheses.
Relationships in the Ca Content Between the Uterine Artery and Other Arteries

To examine whether there were significant correlations between the uterine artery and other arteries with regard to the Ca accumulation, the authors investigated age-related changes of the Ca content in the thoracic and abdominal aortas and the uterine, coronary, common carotid, splenic and common iliac arteries in 14 Japanese women subjects. The subjects ranged in age from 58 to 92 years (average age=82.1±9.2 years). The relationships between the uterine artery and other six arteries were examined with regard to the Ca content. The correlation coefficients between the uterine artery and other arteries in the Ca content were estimated to be 0.098 \((p=0.738)\) for the thoracic aorta, 0.182 \((p=0.534)\) for the abdominal aorta, -0.175 \((p=0.549)\) for the coronary artery, 0.016 \((p=0.956)\) for the common carotid artery, 0.030 \((p=0.920)\) for the splenic artery and 0.271 \((p=0.349)\) for the common iliac artery. No significant correlations were found between the uterine artery and the six arteries with regard to the Ca content. This result suggested that the Ca accumulation in the uterine artery occurred independently of that in the six arteries, such as the thoracic and abdominal aortas and the coronary, common carotid, splenic and common iliac arteries.

DISCUSSION

The present study revealed that the extent of Ca accumulation was different among the four branches of the internal iliac artery and was greater in the uterine arteries in comparison with the other three branches.

There are several reports (Camiel et al., 1967; Fisher and Hamm, 1975; Kadziolka et al., 1985; Punnonen et al., 1995; Crawford et al., 1997) on calcification or atherosclerosis of the uterine artery. Crawford et al. (1997) investigated histological changes of the uterine arteries in both premenopausal and postmenopausal women and reported that 3.4% of the uterine arteries in the premenopausal women contained complex atheromas, whereas 40% of those in the postmenopausal women contained complex atheromas. They revealed that atherosclerosis of the uterine artery appeared to correlate with age. Our finding is consistent with the finding by Crawford et al., (1997).

The present study revealed that Ca accumulation began to occur in the uterine artery of Thai in the forties and increased up to the seventies. The authors (Tohno, Y. et al., 2001a; Tohno, S. et al., 2002) previously investigated age-related changes of elements in the common iliac, internal iliac and coronary arteries of Thai and reported that Ca accumulation began to occur in the common iliac, internal iliac and coronary arteries in the forties. The tendency of Ca accumulation in the uterine arteries of Thai was similar to that in the common iliac, internal iliac and coronary arteries of Thai. However, in Japanese, the uterine artery did not correlate with the thoracic and abdominal aortas and the coronary, common carotid, splenic and common iliac arteries with regard to the Ca content. These results suggested that Ca accumulation occurred in the uterine artery independently of that in the thoracic and abdominal aortas and the coronary, common carotid,
splenic and common iliac arteries. Furthermore, it is unclear whether the uterine artery correlates with the internal iliac artery with regard to the Ca content because it has not yet been investigated.

Regarding the relationships among elements, it was found that there were extremely significant direct correlations among the contents of Ca, P, Mg, Zn and Na in the uterine arteries of both Japanese and Thai. This finding is consistent with the foregoing results obtained in the thoracic aorta and the basilar, coronary, radial, common iliac and femoral arteries (Tohno, Y. et al., 2001b).

It was found that as for the uterine arteries in the subjects more than 60 years of age, the extent of Ca accumulation in the uterine arteries of Thai was one half of that in the uterine arteries of Japanese. The authors previously investigated the differences in age-related changes of elements between the coronary or renal arteries of Japanese and Thai and found that the Ca accumulation occurred at least 10 years earlier and higher in the coronary arteries of Thai in comparison with Japanese (Tohno, S. et al., 2002), whereas the higher Ca accumulation occurred in the renal arteries of Japanese in comparison with Thai at old age (Mahakanukrauh et al., 2005). These results indicated that the uterine artery was similar to the renal artery with regard to age-related changes of the Ca content, but was not similar to the coronary arteries.

Kadziolka et al., (1985) studied the occurrence and characteristics of sclerotic lesions in the uterine arteries of sterile and multiparous pigs and reported that the incidence and degree of sclerotic lesions increased with age and parity.

It is well known that cyclic changes in uterine blood flow occur in association with blood estrogen and progesterone concentrations, and uterine blood flow increases markedly during early pregnancy (Ford, 1982). Konje et al., (2003) investigated the diameter of the proximal uterine artery and uterine artery volume flow during pregnancy by color power angiography and reported that the diameter of the proximal uterine arteries was enlarged about twice during late pregnancy and uterine artery volume flow was increased to 2.5-fold volume.

The birth rate and total fertility rate were high in Japan and Thailand in the 1950’s and 1960’s, when the subjects were still young women. It was thought that the subjects had delivered many babies.

With regard to occurrence of calcification in the uterine artery, there are two possibilities: The first is that the increase of uterine artery blood flow during pregnancy causes mechanical stress for the uterine artery and results in calcification of the uterine artery. The second is that the calcification in the uterine artery occurs with aging, independently of pregnancy or parity. For solving this problem, the authors are planning the study for the analysis of element contents, using the uterine arteries from the subjects with clinical history.
REFERENCES

Application of Concept Mapping to Diabetes Primary Care Planning

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ABSTRACT

The concept mapping has been used in various health issues. However, there was still no application to planning of diabetes care model in primary care setting. The aim of this study was to generate and prioritize diabetes care activities which were continuous, integrated, holistic and involved community participation. The five steps were performed by all stakeholders including health care provider, policy maker, diabetes patient, care giver, health care volunteer and community representative. Firstly, the focus statement was identified as “Identify diabetes care activities which were continuous, integrated, holistic and involved community participation”. Secondly, five-point Likert’s scale was used for rating each activity relative to others in terms of importance and feasibility of each activity. Thirdly, all stakeholders generated, grouped, labeled and prioritized the activities to be the data input. Fourthly, the data were analyzed by multidimensional scaling and hierarchical cluster analysis. Finally, all activities were presented as concept maps. The fifty-four diabetes care activities were generated and grouped into five concepts. They were as follows: 1) providing comprehensive diabetic knowledge; 2) promoting health behavior; 3) setting diabetes management; 4) setting up diabetes care training volunteer (DCTV) and 5) classifying diabetes patient by disease severity, which had average importance values of 4.03, 3.76, 3.73, 3.71 and 3.48, respectively. These activities were prioritized as of relative importance and feasibility with limited barriers in decision-making process. The concept mapping technique was more advantageous in showing the ideas in pictorial form by reliable statistic, however, it could not stimulate creative thinking of stakeholders.

Key words: Concept mapping, Diabetes care planning, Primary care

INTRODUCTION

Diabetes mellitus (DM) is a major chronic disease with a prevalence that is rapidly growing worldwide especially in developing countries (King et al., 1998;
Aekplakorn et al., 2003; Wild et al., 2004). World Health Organization (WHO) estimated that the number of adults with diabetes globally would be doubled by the next twenty years. It was estimated that diabetic patients in 1995 would increase from 135 million to 300 million in 2025 (King et al., 1998). In Thailand, the diabetes prevalence had risen from 2.3% in 1991 to 4.6% in 1996 and 6.9% in 2004 (Ekachampaka, 2008).

The effective diabetes care needs a comprehensive management of health care team approach and multifaceted intervention (Sadur et al., 1999; Renders et al., 2001; Majumdar et al., 2003; Maislos and Weisman, 2004). To provide active participation of multiple stakeholders in diabetes care processes, an effective tool is required to reduce barriers arising from domination of some participants. The difficulty in performing of multiple stakeholders who have different education backgrounds, public health systems, diabetes knowledges, and diabetes care experiences is another obstacle in brainstorming step. In addition, a study found that organizational interventions that facilitated structured and regular review of patients were effective in improving the process care (Renders et al., 2001). However, the quality of diabetes care was still suboptimal to standard of care, especially in community setting (Grant et al., 2005). Many providers in community health centers indicated that enhancement in patient adherence, better health care delivery systems and reform to improve the affordability, accessibility, and efficiency of care are also likely to meet standard of care (Chin, 2001).

The concept mapping or structured conceptualization is a mixed method that combines group processes with a sequence of multivariate statistical analysis (Trochim and Linton, 1986). It takes the ideas of individuals and combines them in specific way to understand how a group thinks about a specific topic. All ideas are organized by multidimensional scaling and hierarchical statistic and displayed in a series of easily-readable concept map. Equality of power in decision making is applied at all steps of the concept mapping so the domination of participants is limited which is an advantage over other tools. The concept mapping has been used in many health issues such as mental illness, alternative medicine, tobacco control program, etc. However, it has not yet been applied in the planning of diabetes care (Galvin, 1979; Trochim and Linton, 1986;Trochim, 1989; Trochim et al., 1994; Trochim, 2003; Baldwin et al., 2004).

Therefore, this study was initiated to serve the equality of stakeholder power in conceptualization of the diabetes care model by the concept mapping. The purpose of the present study was to identify and prioritize diabetes care activities which were continuous, integrated, holistic and involved community participation.

**METHODOLOGY**

**Study settings**

Mitraparb Medical Center (MMC) was purposively selected as a primary care unit (PCU) which meets the standard criteria. It is a contracting unit for primary care (CUP) of Khon Kaen Hospital (KKH) and is located in urban area. It has
been set up in 1999 to provide primary care services which are responsible for registered population under the universal coverage policy, covering 11 communities. There were 13,399 registered residents in 2006. It has been managed as the semi-private clinic under the project of “Warm Community Clinic” since 2004. The majority of finance is supported directly from the National Health Security Office (NHSO). Regarding to the annual reports (2003-2005), diabetes mellitus was the first leading chronic disease with numbers of patients increasing about 30% within two years.

**Study Sample**

Fifteen participants were selected as representatives of each stakeholder by purposive sampling technique. They were a head of community medicine department, seven primary care professionals, four type 2 diabetes patients (2 patients with chronic complications and 2 patients without chronic complications), two community representatives (a head community and a health care volunteer) and a care giver.

**Ethic consideration**

The study was approved by two ethic committees: Khon Kaen Hospital and Khon Kaen University.

**Steps of Concept Mapping Process**

**Step 1: Define a focus statement**

The focus statement was defined by the researcher and then approved by all participants.

**Step 2: Define scale and rating scale**

In planning process, the participants discussed to rate how important and how feasible of each brainstormed item was.

**Step 3: Generate Idea (brainstorming)**

The participants were explained strength and weakness of usual diabetes care at MCC as background information. In addition, they were told the concept mapping process and schedule. After that, they were encouraged to generate a set of statements which ideally should represent the entire conceptual domain for the definite focus statement. Rules of brainstorming process were accepted and the facilitator recorded the ideas as they were generated so that all members of the group could see the set of ideas as they evolved without criticism or discussion of other’s activity except for the purpose of clarification. Audio tape record and photograph were permitted to all participants.

**Step 4: Structuring Idea**

A set of all generated ideas were structured separately by each participant. There were four steps involved. First, each generated idea was printed on a separate index cards (5x5 cm). Second, a complete set of index cards was given to each
participant. All participants were instructed to organize the cards into categories by any implicit criterion as they wanted. Third, they wrote a short phrase, called the ‘concept label’, for each category to describe the characteristic of ideas in each group. Fourth, each idea was rated for its importance and feasibility. The participants were allowed to do the rating at their homes. Each participant finished sorting and rating activity within two weeks.

When each person had completed the sorting task, the results would be combined across people. This was accomplished in two steps. First, the results of the sort for each person were put into a square table or matrix which had fifty-four rows and columns. All of the values of this matrix were either zero or one. A ‘1’ indicated that the activity for that row and column were placed by that person together in a category while a ‘0’ indicated that they were not.

Second, the individually-sorted matrices were added together to obtain a combined group similarity matrix. However, the value in the matrix for any pair of activities indicated how many participants placed that pair of activities together in a pile regardless of what the pile meant to each person or what other statements were or were not in that pile. Values along the diagonal were equal to the number of people who sorted. Thus, in this square group similarity matrix, values could range from zero to the number of people who sorted.

This final similarity matrix was considered to be the relational structure of the conceptual domain because it provided information about how the participants grouped the statements. A high value in this matrix indicated that many of the participants put that pair of activities together in a pile and implied that the activities were conceptually similar in some way. A low value indicated that the activity pair was seldom put together in the same pile and implied that they were conceptually more distinct.

For each statement, one then obtained at least the arithmetic mean of the ratings and sometimes other descriptive statistical information.

**Step 5: Representation Idea**

Sorting data were analyzed by hierarchical cluster analysis, while rating data were analyzed by multidimensional scaling.

**RESULTS**

**Step 1: Define a focus statement:**

The focus statement was defined as “Identifying diabetes care activities which are continuous, integrated, holistic and involved community participation.”

**Step 2: Define scale and rating scale:**

Five-point Likert’s scale was selected for rating each activity relative to other activities in terms of the importance and feasibility of each activity, where 1= relatively unimportant or the least feasible, 2= somewhat important or may be feasible, 3= moderately important or feasible, 4= very important or more feasible
and 5 = extremely important or the most feasible.

**Step 3: Idea generation (brainstorming)**

Fifty-four activities to be the diabetes care model were characterized as continuous, integrated, holistic and involved community participation (Appendix I).

**Steps 4&5: Structuring and representation of idea in concept maps**

- **The point rating map**

The point rating map shows average rating scores across persons for each item. In this study, each activity was rated by its importance and feasibility. The points of importance and feasibility rating map are displayed in Figure 1 and Figure 2, respectively. The number of layer indicated the average importance and feasibility scores. The average data were represented by the layers shown in the upper left corner of each figure. The two maps were represented by the two rating scales in the interpretation form.

![Figure 1: The point of importance rating map.](image)

**Note:** A multiple layer point means average importance value according to legend value (upper left corner). For example: average importance value of activity number 52 (four layers) was between 3.76 and 4.11.

- **The cluster rating map**

When the stakeholders considered point rating maps, they grouped 54-diabetes activities into 5 concepts. They were “providing comprehensive diabetic knowledge”; “promoting health behavior”; “setting diabetes management”; “setting up diabetes care training volunteer (DCTV)”; and “classifying diabetes patient by disease severity”, which had average importance scores of 4.03, 3.76, 3.73, 3.71 and 3.48, respectively (Figure 3).
Considering feasibility rating maps (Figure 4), the “providing comprehensive diabetic knowledge” concept was still the most feasible, and the “classifying diabetes patient by disease severity” concept was considered as the least feasible.

Figure 2: The point of feasibility rating map.

Note: The multiple layer point means average feasibility value according to legend value (upper left corner). For example: average feasibility value of activity number 10 (two layers) was between 2.38 and 2.85.

Figure 3: The cluster importance rating map.
Considering the “setting diabetes management” concept, it showed high average importance score (3.70-3.81) but average feasibility score was the least (3.12-3.21). This meant that it was highly important but was too difficult to practise.

• The pattern matching

Pattern matching is used to compare the patterns of variables across two maps. In this study, the importance rating score was compared between primary care professionals and non-primary care professionals (Figure 5). The results showed that both groups considered the “providing comprehensive diabetic knowledge” concept as the most important and the “classifying diabetes by disease severity” concept as the least important. On the other hand, there were different views regarding “promoting health behavior”, “setting diabetes management” and “setting up diabetes care training volunteers (DCTV)”. Primary care professionals ranked health promotion for diabetes as the second important while non-primary care professionals considered it as the second lowest important. However, the overall relationship between the two groups was still high (r = 0.68).

• Item analysis of rating activities

To examine the relationship between feasibility and importance, two variables of 54 activities were plotted in the scattered graph which was called “the Go-Zone” (Figure 6). The Go-Zone graph assisted the participants to identify areas that should be selected to implement. It was divided into four quadrants, using the axes of the two rating scales of this study. The A, B, C and D quadrants represent high feasibility but low importance; high feasibility and high importance; low feasibility and low importance; and low feasibility but high importance,
respectively. For example, the activity no.161 was rated with high scores in both of the importance and feasibility. It located in quadrant B which implied to high importance and high feasibility activity.

Figure 5: Ladder graph pattern match of primary care professionals and non-primary care professionals on importance rating score.

Figure 6: The Go-Zone.
Note: Quadrant A = low importance but high feasibility, B = high importance and high feasibility, C = low importance and low feasibility, D = high importance but low feasibility

1No.16 activity is “To provide patient understanding in benefit of good and bad control of blood sugar”.
The point map and the cluster map were shown and explained to all participants to further discuss about the maps and summarize the final cluster map. After that, the discussed Go-Zone results and selected 26 activities which located in the quadrant B to be implemented because they were of high importance and high feasibility. The participants also selected other seven activities which located outside the quadrant B but their locations were near the quadrant B and their activities were related to the 26 activities. Finally, 31 diabetic activities were selected to be implemented in the action step (see Appendix II).

**DISCUSSION AND CONCLUSION**

Fifty-four activities were generated and prioritized. They were grouped into five concepts as follows: 1) providing comprehensive diabetic knowledge; 2) promoting health behavior; 3) setting diabetes management; 4) setting up diabetes care training volunteers; and 5) classifying diabetes patient by disease severity, which had average importance values of 4.03, 3.76, 3.73, 3.71 and 3.48, respectively.

As all diabetes care activities were generated by focus group discussion following the concept mapping steps, all ideas were based on the participants’ opinions. This, however, may not cover some activities that all being suggested elsewhere for improving of diabetes care such as psychological or dental aspects. To overcome this limitation, the multiple methods should be conducted for generating more ideas from various stakeholders, using focus group with well designed questionnaire.

In terms of importance and feasibility, the results showed that “providing comprehensive diabetic knowledge” was the main concept and it should be raised in implementation step. The results were similar to the study that applied concept mapping to identify information about techniques and devices generated by the diabetes as reported in this study (Detaillie et. al, 2006). Both diabetes and medical professionals assigned the highest priority to the cluster referring to an employee’s ability to accept and cope with the disease.

The pattern matching confirmed that knowledge and understanding of diabetic disease was recognized from health care professionals and diabetic patients as the most important aspect. On the other hand, the other clusters showed the opposite rating of importance rating score between health care professionals and diabetic patients. “Health promotion for diabetes” was the cluster expressed with the difference of average importance score by both groups. It was rated the second priority by health professionals, but the fourth priority by the diabetes. This might be because health promotion was the activity that did not affect a patient’s health immediately. Most of the patients were more concerned to live from hand to mouth instead of taking care of themselves for disease prevention.

\[^{2}\text{activity no. 1,3,9,11,16,18,19,21,22,24,25,26,28,29,30,31,32,33,34,35,36,37,38,39,41,45}\]

\[^{3}\text{activity no. 5,7,8,17,27,44,53}\]
**Limitation of Study**

In this study, the confusion of participants during the structuring of ideas led to more time consumption compared with other studies (Chin, 2001; Baldwin et al., 2004; Grant et al., 2004). When the usual concept mapping processes take around 15 hours, such time allocation was not enough for this study. So the process was modified by setting up a meeting schedule only twice. The two meetings were set up for generating ideas and the concept map interpretation. The researcher tried to solve the participants’ confusion by extending the duration of sorting and rating of all activities for more than two weeks. Telephone and home visit were also used in reminding and clarifying the sorting, labeling and rating processes.

In spite of these limitations, the concept mapping still provided an effective way in generating understandable findings for nonscientists and clear implication for real practices. The concept mapping is useful in empowering of community and diabetic patients without any barriers.

**ACKNOWLEDGEMENTS**

I would like to thank Prof. William M.K. Trochim who gave the comprehensive knowledge of structured conceptualization, Mitraparb Medical Center staff, all community representatives, diabetes care volunteers and diabetes patients who were my cooperative partners for completeness of this study. Many thanks are given to Graduate School, Khon Kaen University and Thai Health Promotion Foundation for research grant support.

**REFERENCES**


### APPENDICES

**Appendix I:** Fifty-four diabetes care activities with average importance and feasibility that were generated and rated by the participants.

<table>
<thead>
<tr>
<th>Number of Activities</th>
<th>Diabetes care activities</th>
<th>Average Importance</th>
<th>Average Feasibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>providing group education for diabetic patients, particularly appropriate diet control</td>
<td>3.82</td>
<td>3.64</td>
</tr>
<tr>
<td>2</td>
<td>setting up patients meeting once a month at MPCU to share their experiences among patients with optimal blood sugar control and the others</td>
<td>3.55</td>
<td>3.36</td>
</tr>
<tr>
<td>3</td>
<td>regular update diabetic knowledge for diabetic care training volunteers (DCTV)</td>
<td>3.91</td>
<td>3.64</td>
</tr>
<tr>
<td>4</td>
<td>providing first-aid kit for taking care of diabetes patients in communities</td>
<td>3.18</td>
<td>2.91</td>
</tr>
<tr>
<td>5</td>
<td>promoting diabetic screening in high risk group, especially diabetic relatives</td>
<td>3.73</td>
<td>3.73</td>
</tr>
<tr>
<td>6</td>
<td>providing health education for diabetic patients weekly</td>
<td>3.73</td>
<td>3.18</td>
</tr>
<tr>
<td>7</td>
<td>developing diabetic care system by disease severity</td>
<td>3.73</td>
<td>3.73</td>
</tr>
<tr>
<td>8</td>
<td>demonstrating about medication taking per day for individual patients especially in non adherent groups</td>
<td>3.73</td>
<td>3.73</td>
</tr>
<tr>
<td>9</td>
<td>providing medication counseling individually for all diabetic patients</td>
<td>4.36</td>
<td>4.09</td>
</tr>
<tr>
<td>10</td>
<td>monitoring patients diet in their homes</td>
<td>3.09</td>
<td>2.73</td>
</tr>
<tr>
<td>11</td>
<td>providing comprehensive diabetic care including screening, education, treatment, monitoring chronic complications, and home care visits</td>
<td>4.45</td>
<td>3.82</td>
</tr>
<tr>
<td>12</td>
<td>classification of diabetic patients by disease severity for appropriate treatment</td>
<td>3.64</td>
<td>3.36</td>
</tr>
<tr>
<td>13</td>
<td>providing exercise demonstration in communities every week</td>
<td>3.91</td>
<td>3.09</td>
</tr>
<tr>
<td>14</td>
<td>giving advice about appropriate food taking to individual</td>
<td>3.64</td>
<td>3.45</td>
</tr>
<tr>
<td>15</td>
<td>extending office hours for general patient in the afternoon or in the evening</td>
<td>3.36</td>
<td>3.27</td>
</tr>
<tr>
<td>16</td>
<td>providing patient understanding in benefits of good blood sugar control and effects of bad control</td>
<td>4.45</td>
<td>4.27</td>
</tr>
<tr>
<td>17</td>
<td>setting up DCTV to be community representatives who would provide moral support and remind patients to see doctors</td>
<td>3.73</td>
<td>3.09</td>
</tr>
<tr>
<td>18</td>
<td>setting up to regularly monitor eye and foot complication</td>
<td>4.09</td>
<td>3.73</td>
</tr>
<tr>
<td>19</td>
<td>setting up diabetic care management as standard of MOPH</td>
<td>4.27</td>
<td>3.64</td>
</tr>
<tr>
<td>20</td>
<td>providing herbal knowledge by performing collaboration among DCTV, health volunteers and primary care professionals</td>
<td>3.36</td>
<td>3.18</td>
</tr>
</tbody>
</table>
### Describing Effects of Low and High Blood Sugar, and How to Cope with It

<table>
<thead>
<tr>
<th>No.</th>
<th>Activity</th>
<th>Rating by Nurses</th>
<th>Rating by Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>describing effects of low and high blood sugar, and how to cope with it</td>
<td>4.27</td>
<td>4.00</td>
</tr>
<tr>
<td>22</td>
<td>providing proactive home care visit and close monitoring in case of high risk to diabetic complications</td>
<td>4.45</td>
<td>3.64</td>
</tr>
<tr>
<td>23</td>
<td>visiting other primary care settings to learn how to be effective diabetic care management</td>
<td>2.91</td>
<td>2.27</td>
</tr>
<tr>
<td>24</td>
<td>checking fasting blood sugar before 8.00 AM at diabetic clinic</td>
<td>3.82</td>
<td>3.36</td>
</tr>
<tr>
<td>25</td>
<td>demonstrating and preparing diabetes diet for patients every week</td>
<td>3.82</td>
<td>3.64</td>
</tr>
<tr>
<td>26</td>
<td>admiring diabetic patients who can control blood sugar, and exchange their experience with others</td>
<td>4.27</td>
<td>4.09</td>
</tr>
<tr>
<td>27</td>
<td>building up a multidisciplinary team by cooperative setting of therapeutic plan and monitoring diabetic patients</td>
<td>3.73</td>
<td>3.45</td>
</tr>
<tr>
<td>28</td>
<td>Setting up DCTV in each community (at least one volunteer per community)</td>
<td>3.82</td>
<td>3.36</td>
</tr>
<tr>
<td>29</td>
<td>Setting up a DCTV monitoring book record to regularly monitor patients and provide continuity record</td>
<td>3.91</td>
<td>3.73</td>
</tr>
<tr>
<td>30</td>
<td>counseling proper exercise to individual patients</td>
<td>4.18</td>
<td>3.73</td>
</tr>
<tr>
<td>31</td>
<td>Monitoring and advising DCTV on their duties continuously</td>
<td>3.91</td>
<td>3.55</td>
</tr>
<tr>
<td>32</td>
<td>strengthening diabetic patients to participate in diabetic prevention activities and promote diabetic screening</td>
<td>4.09</td>
<td>3.73</td>
</tr>
<tr>
<td>33</td>
<td>providing a spiritual room for psychological counseling in diabetic patients with mental problems such as stress, anxiety etc.</td>
<td>3.45</td>
<td>3.18</td>
</tr>
<tr>
<td>34</td>
<td>emphasizing activities to improve quality of life such as exercise, foot care, appropriate diet, recreation of primary care professionals and diabetic patients</td>
<td>4.18</td>
<td>3.73</td>
</tr>
<tr>
<td>35</td>
<td>setting up to regularly monitor system for home care visit in discharge patients</td>
<td>3.91</td>
<td>3.55</td>
</tr>
<tr>
<td>36</td>
<td>providing group education emphasizing on how to detect abnormal symptoms and serious diabetic complications</td>
<td>4.36</td>
<td>4.27</td>
</tr>
<tr>
<td>37</td>
<td>fixing two staff members of primary care professionals who are responsible for diabetic patients</td>
<td>3.91</td>
<td>3.73</td>
</tr>
<tr>
<td>38</td>
<td>updating diabetic database for effective care and monitoring</td>
<td>4.45</td>
<td>4.09</td>
</tr>
<tr>
<td>39</td>
<td>providing diabetic care at home in case of handicapped patients</td>
<td>3.82</td>
<td>3.36</td>
</tr>
<tr>
<td>40</td>
<td>providing transportation service for diabetic patient who must go to wound dressing every day</td>
<td>3.00</td>
<td>2.00</td>
</tr>
<tr>
<td>41</td>
<td>determining appropriate number of patients for each clinic visit</td>
<td>3.82</td>
<td>4.09</td>
</tr>
<tr>
<td>42</td>
<td>revising the follow up system for each community</td>
<td>3.45</td>
<td>3.18</td>
</tr>
<tr>
<td>43</td>
<td>educating care givers about patients care at home</td>
<td>3.91</td>
<td>2.82</td>
</tr>
</tbody>
</table>
Appendix 2: Thirty-three activities were selected for implementation.

<table>
<thead>
<tr>
<th>Concept 1: Comprehensive diabetic knowledge</th>
<th>Diabetes care activities</th>
<th>Average Importance</th>
<th>Average Feasibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 Promoting patient understanding in benefits of good</td>
<td>4.45</td>
<td>4.27</td>
<td></td>
</tr>
<tr>
<td>blood sugar control and negative effects of its bad control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 Providing group education emphasizing on how to detect abnormal symptoms and serious diabetic complications</td>
<td>4.36</td>
<td>4.27</td>
<td></td>
</tr>
<tr>
<td>9 Providing medication counseling individually for all diabetic patients</td>
<td>4.36</td>
<td>4.09</td>
<td></td>
</tr>
<tr>
<td>21 Describing effects of low and high blood sugar, and how to cope with it</td>
<td>4.27</td>
<td>4.00</td>
<td></td>
</tr>
<tr>
<td>26 Admiring diabetic patients who can control blood sugar, and exchange their experiences with others</td>
<td>4.27</td>
<td>4.09</td>
<td></td>
</tr>
<tr>
<td>30 Counseling proper exercise to individual patients</td>
<td>4.18</td>
<td>3.73</td>
<td></td>
</tr>
<tr>
<td>45 Educating diabetic patients and their families by providing leaflets</td>
<td>3.82</td>
<td>3.64</td>
<td></td>
</tr>
<tr>
<td>34 Emphasizing activities to improve quality of life such as exercise, foot care, appropriate diet, recreation of primary care professionals and diabetic patients</td>
<td>4.18</td>
<td>3.73</td>
<td></td>
</tr>
<tr>
<td>32 Strengthening diabetic patients to participate in diabetic prevention activities and promote diabetic screening</td>
<td>4.09</td>
<td>3.73</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concept 2: Health promotion for diabetes</th>
<th>Diabetes care activities</th>
<th>Average Importance</th>
<th>Average Feasibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>34 Emphasizing activities to improve quality of life such as exercise, foot care, appropriate diet, recreation of primary care professionals and diabetic patients</td>
<td>4.18</td>
<td>3.73</td>
<td></td>
</tr>
<tr>
<td>32 Strengthening diabetic patients to participate in diabetic prevention activities and promote diabetic screening</td>
<td>4.09</td>
<td>3.73</td>
<td></td>
</tr>
</tbody>
</table>
### Concept 3: Diabetes management in primary care unit

<table>
<thead>
<tr>
<th>No.</th>
<th>Activity Description</th>
<th>Score</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>Updating diabetic database for monitoring and having more effective care</td>
<td>4.45</td>
<td>4.09</td>
</tr>
<tr>
<td>11</td>
<td>Providing comprehensive diabetic care including screening, education, treatment, monitoring chronic complications, and home care</td>
<td>4.45</td>
<td>3.82</td>
</tr>
<tr>
<td>19</td>
<td>Setting up diabetic care management following the standard of MOPH</td>
<td>4.27</td>
<td>3.64</td>
</tr>
<tr>
<td>18</td>
<td>Setting up regular monitoring system for eye and foot complications</td>
<td>4.09</td>
<td>3.73</td>
</tr>
<tr>
<td>35</td>
<td>Setting up regular monitoring system for home care in discharged patients</td>
<td>3.91</td>
<td>3.55</td>
</tr>
<tr>
<td>37</td>
<td>Fixing two staff members of primary care professionals who are responsible for diabetic patients</td>
<td>3.91</td>
<td>3.73</td>
</tr>
<tr>
<td>39</td>
<td>Providing diabetic care at home for handicapped patients</td>
<td>3.82</td>
<td>3.36</td>
</tr>
<tr>
<td>53</td>
<td>Providing annual diabetic screening in community</td>
<td>3.82</td>
<td>3.27</td>
</tr>
<tr>
<td>41</td>
<td>Determining appropriate number of patients for each clinic visit</td>
<td>3.82</td>
<td>4.09</td>
</tr>
<tr>
<td>24</td>
<td>Checking fasting blood sugar before 8.00 AM at diabetic clinic</td>
<td>3.82</td>
<td>3.36</td>
</tr>
<tr>
<td>27</td>
<td>Building up a multidisciplinary team by cooperative setting of therapeutic plan and monitoring diabetic patients</td>
<td>3.73</td>
<td>3.45</td>
</tr>
</tbody>
</table>

### Concept 4: Community participation by setting up DCTV

<table>
<thead>
<tr>
<th>No.</th>
<th>Activity Description</th>
<th>Score</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Educating regular update of diabetic knowledge for DCTV</td>
<td>3.91</td>
<td>3.64</td>
</tr>
<tr>
<td>31</td>
<td>Monitoring and advising DCTV on their duties continuously</td>
<td>3.91</td>
<td>3.55</td>
</tr>
<tr>
<td>29</td>
<td>Setting up a DCTV monitoring book record to regularly monitor patients and provide continuity record</td>
<td>3.91</td>
<td>3.73</td>
</tr>
<tr>
<td>25</td>
<td>Demonstrating and preparing diabetes diet for patients every week</td>
<td>3.82</td>
<td>3.64</td>
</tr>
<tr>
<td>28</td>
<td>Setting up diabetic training volunteers in each community (at least one volunteer per community)</td>
<td>3.82</td>
<td>3.36</td>
</tr>
<tr>
<td>17</td>
<td>Setting up diabetic care volunteers to be community representatives who provide moral support and being a patient’s reminders for the doctor appointments</td>
<td>3.73</td>
<td>3.09</td>
</tr>
<tr>
<td>44</td>
<td>Providing diabetic knowledge to diabetic patients, care givers, and DCTV</td>
<td>3.73</td>
<td>3.27</td>
</tr>
</tbody>
</table>

### Concept 5: Classification of diabetes patient by disease severity

<table>
<thead>
<tr>
<th>No.</th>
<th>Activity Description</th>
<th>Score</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>Providing proactive home care and having closer monitoring in case of high risk to diabetic complications</td>
<td>4.45</td>
<td>3.64</td>
</tr>
<tr>
<td>7</td>
<td>Developing diabetic care system by disease severity</td>
<td>3.73</td>
<td>3.73</td>
</tr>
<tr>
<td>12</td>
<td>Classification of diabetic patients by disease severity for appropriate treatment</td>
<td>3.64</td>
<td>3.36</td>
</tr>
</tbody>
</table>

Note: DCTV means Diabetes Care Training Volunteer
Predicting Factors of Dependent Care Behaviors among Mothers of Toddlers with Congenital Heart Disease

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ABSTRACT

The alteration of the hemodynamic pattern caused by congenital heart disease (CHD) can make the affected children be at risk of morbidity and mortality. Care of mothers is particularly important for toddlers with un-repaired CHD, as the toddlers rely on their mothers for taking medication, feeding and monitoring of complications. With guidance from the Self-Care Deficit Nursing Theory, this study aimed to describe the relationships between dependent care behaviors among mothers of toddlers with CHD and parenting stress, perceived social support, perceived self-efficacy, CHD knowledge, educational background and family income. Also, the abilities of those study variables in predicting dependent care behaviors of the mothers were identified. A total of 95 participants were enrolled into the study. When the effects of other variables were controlled, the results showed that perceived self-efficacy and family income were positively correlated with maternal dependent care behaviors (r = .62, p < .01; r = .21, p < .05, respectively). Importantly, perceived self-efficacy was the only predictor accounting for 43.80 % of the variance in the mothers’ dependent care behaviors. Thus, building self-efficacy is likely to be a reasonable starting point for interventions aiming to enhance dependent care behaviors in mothers of toddlers with CHD.

Key words: Dependent care behaviors, Congenital heart disease, Toddler, Predicting factors

INTRODUCTION

The CHD with increased pulmonary blood flow, for example, ventricular septal defect (VSD), atrial septal defect (ASD) or patent ductus arteriosus (PDA), permits blood to pass between the systemic and pulmonary circulation through an abnormal opening. This condition might result in symptoms of congestive heart failure (Wong et al., 2001), respiratory tract infection (Bhatt et al., 2004) and
growth failure (Chen et al., 2004) that are associated with increased morbidity and mortality. Generally, surgical intervention is the treatment of choice for most CHD, but in Thailand, waiting time for cardiac surgery among CHD children has been reported to be as long as approximately six months (Khongphatthanayothin et al., 2005). The emergence of a group of unrepaired CHD has heightened the need of attention, especially to the children who are waiting for surgical intervention.

As surgical treatment for Thai CHD children is usually performed in preschoolers (Khongphatthanayothin et al., 2005), the nature of disease and developmental stages have placed mothers of the toddlers in a crucial position to keep the child’s health as normal as possible before surgery. However, one study has shown that care behaviors of mothers for toddlers with CHD were at moderate level (Chatrum, 2003). Studies concentrating on oral health care for CHD children also indicated that care of parents failed below the recommended activities for the child’s care needs (Saunders and Roberts, 1997; Kongsrichareon et al., 2002; Silva et al., 2002). Since there is only a few literature focusing on care behaviors of mothers of toddlers with CHD, knowledge in this area still remains to be fulfilled.

Based on the Self-Care Deficit Nursing Theory (Orem, 2001), mothers function as dependent care agents who perform self-care on behalf of their children in maintaining life and health. Individuals who engage in dependent care are assumed to have abilities (dependent care agency) to meet requirements of the dependents. Dependent care behavior is affected by dependent care agency and the basic conditioning factors. Hence, the Orem’s theory might be useful to explain care behaviors and associated factors among mothers of toddlers with CHD.

In CHD literature, relationships were found between care behaviors of the mothers and maternal age, education, family income, accurate perception of disease (Azumpinzub, 1997) and perception of health of children with CHD (Chotibang et al., 2001). Findings from clinical trials also demonstrated influence of self-efficacy (Chottivivatayatarakorn, 2000), social support (Dulyakasem, 1993) and perception of CHD and social support (Kamproh, 2001) on care behaviors of the mothers. Nevertheless, little is known about the most important factors and ability of them in explaining variation of the mothers’ care behaviors. In addition, previous studies have reported the stressful impact of being parents of children with CHD (Pelchat et al., 1999; Uzark and Jones, 2003; McGrath and Kolwaite, 2006). Parents with higher education were more likely to have greater knowledge related to CHD (Beeri et al., 2001; Cheuk et al., 2004). Based on the existing evidence, research is needed to explore in greater depth regarding care behaviors of the mothers, in particular for toddlers with ventricular septal defect (VSD), atrial septal defect (ASD) or patent ductus arteriosus (PDA) who have not had surgery. Also, the potential predictor of care behaviors among mothers of toddlers with CHD is crucial to be defined because it would be useful to guide appropriately interventions to enhance the mothers’ care quality.

With the guiding of Orem’s theory (2001), some variables were selected to examine for their influences and ability in prediction of dependent care be-
haviors among mothers of toddlers with CHD. The variables including parenting stress, perceived social support, educational background and family income were considered as basic conditioning factors that would affect maternal dependent care behaviors in Orem’s perspective. Knowledge of CHD is inherent in dependent care agency that will aid mothers to understand, judge and make decision about dependent care actions. In addition, perceived self-efficacy is linked to the transitional capability of dependent care operations in dependent care agency because this variable plays an important part in judgment of the mothers about their capacity to perform dependent care behaviors in order to produce desired outcomes.

Objectives of the Study

This study aimed to examine the relationships between dependent care behaviors among mothers of toddlers with CHD and parenting stress, perceived social support, educational background, family income, CHD knowledge and perceived self-efficacy. Also, the abilities of those study variables in predicting dependent care behaviors of the mothers were identified.

MATERIALS AND METHODS

This study was a correlational design. A sample was selected using purposive sampling method. The sample consisted of 95 mothers of children aged 1-3 years diagnosed with VSD or ASD or PDA who accompanied their children to attend pediatric cardiology clinic at two public tertiary hospitals in Chiang Mai and Phitsanulok. The participants were structurally interviewed using the Demographic Data Form, the Thai version of the Parenting Stress Index-Short Form (PSI-SF), the Personal Resource Questionnaire (PRQ-85- Part II), the CHD Knowledge Scale, the Dependent Care Behaviors in Mothers of Toddlers with CHD Scale and the Maternal Perceived Self-efficacy Scale. Descriptive statistics were used to describe samples with respect to individual information. Stepwise multiple regression analysis was run to examine multiple correlations.

RESULTS

Demographic characteristics of the 95 participants revealed that the age range of participants was 18 to 45 years, with a mean age of 30.51 years. The majority of them (38.95%) achieved secondary school certificates or diploma. The average family income was 11,895.89 Baht/month. With regard to the characteristic of family, the majority of participants (56.84 %, n = 54) had extended family. Approximately three-fourths of the toddlers with CHD in this study were diagnosed with VSD (71.58 %, n = 68), one-sixth with PDA (14.74 %, n = 14) and another one-sixth with ASD (13.68 %, n = 13). Approximately one-third of them (33.68 %, n = 32) were taking medications related to CHD. During the past 3 months, more than half of the samples had respiratory tract infection (RI) at least 1 time (63.16 %, n = 60), only a few had cyanosis (2.10 %, n = 2) and none of them had edema. Approximately one-third of them needed admission to the hospital at
least 1 time (30.53 %, n = 29).

**Table 1.** Correlation matrix of all study variables (n = 95).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Care beh.</th>
<th>Parenting stress</th>
<th>Social support</th>
<th>Self-efficacy</th>
<th>Knowledge</th>
<th>Edu.</th>
<th>Income</th>
</tr>
</thead>
<tbody>
<tr>
<td>Care beh.</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parenting stress</td>
<td></td>
<td>-.21*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social support</td>
<td></td>
<td></td>
<td>.33**</td>
<td>-.33**</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-efficacy</td>
<td></td>
<td></td>
<td></td>
<td>.66**</td>
<td>-.40**</td>
<td>.44**</td>
<td>1.00</td>
</tr>
<tr>
<td>Knowledge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Edu.</td>
<td></td>
<td></td>
<td>.08</td>
<td></td>
<td>.16</td>
<td>.13</td>
<td>.21*</td>
</tr>
<tr>
<td>Income</td>
<td></td>
<td></td>
<td>-.24**</td>
<td></td>
<td>.21*</td>
<td>.05</td>
<td>.07</td>
</tr>
</tbody>
</table>

* p < .05, ** p < .01

**Note:** Care beh. = dependent care behaviors in mothers of children with CHD; Social support = Perceived social support; Self-efficacy = Perceived maternal self-efficacy; Knowledge = CHD knowledge; Edu. = Educational background; Income = Family income

Significant bivariate correlations were found between dependent care behaviors of the mothers and perceived self-efficacy (r = .66, p < .01), perceived social support (r = .33, p < .01), and parenting stress (r = -.21, p < .05) (Table 1). Since the intercorrelations were found among the study variables, therefore, simultaneous regression was performed to examine the partial correlation coefficient or the correlation of a study variable and dependent care behaviors when the effects of other variables were controlled. As shown in Table 2, partial correlation coefficient between dependent care behaviors and perceived self-efficacy was a highly significant positive relationship (r = .62, p < .01). Moreover, a low significant relationship was found between dependent care behaviors and family income (r = .21, p < .05). Importantly, perceived self-efficacy was the only predictor accounting for 43.80 % of the variance in the mothers’ dependent care behaviors (Table 3).

**Table 2.** Coefficient correlations of dependent care behaviors in mothers of children with CHD and all study variables (n = 95).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Partial correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parenting stress</td>
<td>.14</td>
</tr>
<tr>
<td>Perceived social support</td>
<td>.05</td>
</tr>
<tr>
<td>Perceived self-efficacy</td>
<td>.62**</td>
</tr>
<tr>
<td>Knowledge of CHD</td>
<td>-.18</td>
</tr>
<tr>
<td>Educational background</td>
<td>-.12</td>
</tr>
<tr>
<td>Family income</td>
<td>.21*</td>
</tr>
</tbody>
</table>

* p < .05, ** p < .01
Table 3. Predicting factor of maternal dependent care behaviors (n= 95).

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>SE B</th>
<th>β</th>
<th>t value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>1.407</td>
<td>.134</td>
<td>10.462**</td>
<td></td>
</tr>
<tr>
<td>Perceived self-efficacy</td>
<td>.008</td>
<td>.001</td>
<td>.662</td>
<td>8.511**</td>
</tr>
</tbody>
</table>

$R^2 = .438$, Adjusted $R^2 = .432$, $F (1, 93) = 72.433$, ** $p < .01$

DISCUSSION

Through the interpretation of the partial correlation that was found in this sample, perceived self-efficacy and family income indicated influences on the dependent care behaviors of mothers of toddlers with CHD. The mothers who had high perceived self-efficacy and high family income also had better maternal dependent care behaviors. This finding is significant since the perceived self-efficacy which was conceptualized as a transitional capability of dependent care operations in dependent care agency had a high relationship with maternal dependent care behaviors. In keeping with Orem’s theory (2001), the transitional capability of self/dependent-care is cognitive process such as thinking, judging and deciding about self/dependent-care situation before self/dependent-care action is performed. For individuals who perceived that they have ability for self/dependent-care or self-efficacy, this situation will end with carrying out self/dependent-care action. This finding is consistent with other studies reporting a relationship between self-efficacy and maternal care behaviors (Cluskey, 1999; Seo, 2003; Jackson and Scheines, 2005).

According to Orem (2000), resources availability and adequacy affect the means to meet self-care requisites and the associated care measures. In the present study, family income was significantly related with dependent care behaviors of mothers for toddlers with CHD. Thus, one possible explanation for the existence of significant relationship between family income and maternal dependent care behaviors may be that mothers with higher income, compared to those with limited income, find it less difficult to afford healthier food options, healthcare services, accommodation, as well as utilities for their child. Especially, previous research also supported the relationship between family income and maternal childcare behaviors (Azumpinzub, 1997; Ronsaville and Hakim, 2000; Iram and Butt, 2004).

In the present study, perceived self-efficacy was the only predictor accounting for 43.80% of the variance in the mothers’ dependent care behaviors for toddlers with CHD. This finding is somewhat consistent with the findings from the previous studies that showed perceived self-efficacy is predictive of maternal care behaviors such as providing an environment that enhances intellectual and emotional development (Jackson and Scheines, 2005), discipline style (Sanders and Woolley, 2005) and parental involvement and monitoring (Shumow and Lomax, 2002). More specifically, results of the current study provided strong support that perceived self-efficacy leads to a better dependent care behavior. In light of this evidence, the result of this study supports the emphasis of interventions aimed to
increase the maternal self-efficacy.

Even though a statistically significant positive correlation was found between family income and dependent care behaviors in the current study, results from regression analyses did not show significant effect of family income in predicting dependent care behaviors of the mothers. This result may be partly due to a small magnitude of relationship at a marginal level of significance between family income and dependent care behaviors. When stepwise multiple regression was used to determine effective predictors, the variable with the greatest contribution is added first. Then, the next variables are selected for inclusion, based on their incremental contribution over the variable(s) already in the equation (Hair et al., 1998). Thus, what family income accounted for the variance in the dependent care behaviors was so small and unable to capture any significant effect.

In conclusion, care for children with CHD is demanding and there is now sufficient evidence that perceived self-efficacy predicts much variance in dependent care behaviors in mothers of toddlers with CHD. Intervention programs that focus on strengthening maternal perceived self-efficacy can be recommended as a method to promote the mothers’ care behaviors. More research is needed to test the mediator and moderator effects of the study variables. In addition, future investigations with a sample of children with similar CHD severity would also allow for more refined designs.

ACKNOWLEDGEMENTS

The authors would like to express gratitude to Thailand Nursing Council for providing partial financial support for this study. Special thanks for collaboration go to Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Buddhachinaraj Hospital, Phitsanulok and all participants in this study.

REFERENCES


An Internet-Based Program to Promote Healthy Eating Behavior among Thai Early Adolescents

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ABSTRACT

This participatory action research (PAR) aimed to develop an Internet-based program for promoting healthy eating behavior among Thai early adolescents based on a participatory approach. The study participants were 73 adolescent members and 27 adolescent leaders, aged 12-13 years, attending a private school in the urban area, Chiang Mai, Thailand. Key stakeholders were also involved, including: fifteen teachers, one school nurse and seven parents. The study used various methods to collect both qualitative and quantitative data. The findings of this study are presented in respect of unhealthy eating behavior issues among Thai early adolescents, critical elements of the Internet-based program and the outcomes of implementing the Internet-based program.

Key words: Early adolescents, Healthy eating behavior, An Internet-based program, Participatory action research, Thailand

INTRODUCTION

Thailand is similar to many other countries where unhealthy eating behavior is an important problem that affects the nutritional status during adolescence, including overweight and obesity, undernutrition and micronutrient deficiencies and eating disorder. Several studies have shown that adolescents in Bangkok and other urbanized provinces not only consume poor energy foods and skip meals, but also consume high amounts of fast and energy-dense foods, saturated fat and dietary supplement products (Limpijarnkit, 1995; Anukoolwuthipong, 1997; Boonpraderm, 1997; Pawaputanond Na Mahasarakam, 2001; Phuphaibul et al., 2003). Ensuring good nutrition is challenging for adolescents because this developmental stage is the peak time for body image dissatisfaction, with many teens expressing a desire to have a body weight less than their present weight, and therefore liable to misuse drugs and food products for weight loss (Gunta, 2002; Tanausawanont, 2006). Food habits, lifestyles and social behavior established during adolescents are highly predictable to contribute to poor nutrition and increased diseases in adulthood.
Innovative and effective strategies are needed to promote healthy eating behavior among adolescents, particularly in urban areas. Adolescents are typically early adopters of new technologies. The increasing availability of information technology creates innovative channels for health promotion (Skinner et al., 1997). The Internet in particular provides unique opportunities for engaging youth (Skinner et al., 2003). The Internet can serve as an important tool in acquiring health information because adolescents can easily access this medium (Borzekowski and Rickert, 2001). Using computer-based instruction provides increased learner control, independence and decision-making, making it an effective method of instruction sensitive to the learning preferences of youth (Shegog et al., 2001; Long and Stevens, 2004). Some studies have shown that the interventions available through the Internet and computer-assisted instructional programs are effective in promoting self-efficacy for healthy behaviors in adolescents and other groups (Anderson et al., 2001; Shegog et al., 2001; Long and Stevens, 2004). In addition, some researchers report that healthy eating interventions that use Web-based nutrition education for adolescents results in significant reduction in fat consumption and decreased body fat (Tate et al., 2001; Frenn et al., 2003; Frenn et al., 2005; Williamson et al., 2005; Long et al., 2006).

These previous studies suggest that the Internet is an innovative and effective method to change health-related behavior and improve health outcomes among adolescents. However, Web-based programs are typically designed without adequate input from adolescent perspectives. According to Skinner et al., (1997), adolescents should be integrally involved in all stages of design, development, evaluation and dissemination of developed Internet-based program.

Recognizing the benefit of using a participatory approach, a study was designed to develop an Internet-based program to enhance knowledge and promote eating behavior change among Thai early adolescents at a private school in the urban area, Chiang Mai, Thailand. Following the principles of participatory action research, the development would include key stakeholders, and be based upon the adolescents' needs and desires. An additional goal is that the process would serve to increase capacity building and empowerment of adolescents. In addition, the process of the study aims to create, within the target group, a sense of ownership of the program which would lead to sustainable change in the future.

**Objectives of the Study**

The overall goal of this study was to develop an Internet-based program that promotes healthy eating behavior among Thai early adolescents at a private school in the urban area, Chiang Mai, Thailand, based on a participatory approach. The objectives in this study were as follows:

1. To identify issues that teachers, parents and early adolescents report as promoting unhealthy eating behavior in Thai early adolescents.
2. To identify critical elements of an Internet-based program to promote healthy eating behavior that is culturally appropriate to Thai early adolescents.
3. To develop and implement an Internet-based program to promote healthy eating behavior in Thai early adolescents using a participatory approach.
4. To evaluate the outcomes of implementing an Internet-based program that promotes healthy eating behavior in Thai early adolescents based on a participatory approach.

**MATERIALS AND METHODS**

**Research Design**

Participatory action research (PAR), an enhancement approach, was considered to be appropriate for this study.

**Setting and Participants**

The research took place with a purposive sample from one private school in an urban area in Chiang Mai province, Thailand. This school was selected because it is a big private school located in an urban area where fast-food restaurants and other convenience food stores are available, and as the students’ families are of high socioeconomic status, unhealthy food is easily accessible to adolescents. In addition, this school has the availability of the Internet Server to support the development of an Internet-based program.

Research participants in this study were 100 early adolescents, both male and female, aged 12-13 years, comprising two groups, made up of seventy-three adolescent members and twenty seven adolescent leaders. Other stakeholders involved in this study as facilitators were fifteen teachers, one school nurse and seven adolescents’ parents.

**Data Collection**

Both qualitative and quantitative data were collected using various methods. Qualitative data were collected through participatory activity, group discussions, group meetings and participant observations. Additional quantitative data were collected through a demographic data sheet, a test of knowledge in adolescence’s food consumption, an attitude to food consumption questionnaire, a food consumption behavior questionnaire, a nutritional status assessment tool and an Internet-based program satisfaction questionnaire.

**Data Analysis**

Quantitative data were analyzed by using descriptive statistics, Wilcoxon signed-rank test, paired t-test and chi-square test. Qualitative data were analyzed using content analysis.

**Research Process**

The PAR process in this study was based on the basic action research process - “look, think, act”, as outlined by Stringer (1999). The PAR process extended over a period of sixteen months, from May 2007 to August 2008, divided into the following eight steps:

*Step 1 Establishing collaboration.* The first step of the PAR process aimed to establish a relationship with school administrators and teachers to obtain permis-
sion and their support for this project. The researcher met the school administrators and teachers in order to present the objectives, research processes and potential benefits to the school.

**Step 2 Recruiting adolescent participants and other stakeholders.** The second step aimed to recruit adolescent participants and facilitators (teachers, a school nurse and parents) who would be interested in participating in this study. Recruitment strategies included flyers which provided the information about the research objectives and recruiting criteria for the students (grades 7-8), teachers who taught the students in grades 7-8, a school nurse and adolescent parents. Informed consent was obtained from adolescent participants and other stakeholders as well.

**Step 3 Assessing eating behavior issues and needs.** The third step aimed to identify unhealthy eating behavior issues and potential strategies for promoting healthy eating behavior. The researcher developed a set of activities to conduct need assessment for each group through the reflection process used during participatory activities in the group of early adolescents and group discussions with both teachers’ and parents’ groups. These activities enabled participants to express and review their experience of unhealthy eating behavioral issues in Thai early adolescents as well as express their opinions on the potential critical elements of an Internet-based program for promoting healthy eating behavior among Thai early adolescents.

**Step 4 Recruiting and preparing adolescent leaders.** The fourth step aimed to strengthen adolescent leaders’ capacities to be competent leaders for developing an Internet-based program and disseminating knowledge regarding healthy eating behavior to other adolescents in the school. The researcher recruited twenty-seven adolescent leaders from early adolescents who volunteered to be adolescent leaders. Then the researcher set up a training session or workshop based on the adolescent leaders’ needs, with the aim of improving the working efficiency of the research stakeholders’ team and also to strengthen the leadership skills and teamwork spirit. This workshop or training session was arranged at the school for one and a half days. The activities in this workshop were based on a successful program used to train youth leaders in HIV/AIDS prevention, a patented-right program developed by Youth Family and Community Development, the Faculty of Nursing, Chiang Mai University (Fongkaew et al., 2007).

**Step 5 Planning and developing the Internet-based program.** The fifth step aimed to set up the tentative plan for developing an Internet-based program; and to develop an Internet-based program and research instruments for evaluation of the outcomes of the program. This step consisted of four activities:

**Activity I: Organizing reflection session on eating behavior issues and needs data.** The researcher conducted the group meeting to brief the adolescent leaders about the findings from assessing eating behavior issues and needs. At this meeting, the adolescent leaders were encouraged to share their opinions and reflect upon the obtained data.
Activity II: Planning the program. The researcher encouraged the adolescent leaders to express their opinions during brainstorming session for planning the program. The adolescent leaders proposed that the tasks could be shared among them based on their abilities and expertise. The responsible leaders of subgroups had to perform their designated duties, so a tentative working schedule appeared, specifying the beginning and ending of the schedule, including designation of the consulting teachers for supervision and advice about the tasks that involved one health education teacher, one school nurse and two computer teachers.

Activity III: Developing the Internet-based program. The adolescent leaders created the six critical components of the Internet-based program, which included contents or information for promoting healthy eating behavior, video clips, animations, webboard discussions, a game and quiz exercises.

Activity IV: Developing the research instruments in collaboration with adolescent leaders. The researcher shared the knowledge about the existing instruments regarding eating behavior in adolescents that had been developed from other researchers, including a test of knowledge in adolescence’s food consumption, an attitude to food consumption questionnaire and a food consumption behavior questionnaire. The adolescent leaders were then encouraged to discuss and share their thoughts about the methods and instruments used for evaluating the outcomes of implementing the program. As a result, these developed research instruments were revised based on feedback and made appropriate to evaluate the outcomes of implementing the program. These research instruments were then tested for validity and reliability by sending them to five experts and testing with thirty early adolescents.

Step 6 Implementing the Internet-based program. The sixth step aimed to implement an Internet-based program for promoting healthy eating behavior in collaboration with adolescent leaders. Before implementing the program, the adolescent leaders in collaboration with the researcher assessed baseline data of the adolescent participants (27 adolescent leaders and 73 adolescent members) including their knowledge of food consumption, attitudes towards food consumption, eating behavior and nutritional status (weight for height). The implementing program was arranged to last approximately 12 weeks. During this step, the adolescent members and the adolescent leaders were able to access the Internet-based program at the school and outside the school wherever the Internet was available for access.

The process of implementing the Internet-based program for promoting healthy eating behavior in the present study covered three components as follows:

Component I: Encouraging teamwork and the involvement of adolescent leaders. This component was composed of four crucial strategies including: 1) strengthening leadership skills and the teamwork; 2) brainstorming to set up the action plan of implementing the program; 3) brainstorming to identify methods to motivate adolescent members using the program; and 4) brainstorming to identify
methods for evaluation of the outcomes.

Component II: Maximizing the use of the Internet-based program. This component was comprised of two strategies, namely: 1) motivating and encouraging regularity in using the program; and 2) encouraging self-directed learning and sharing knowledge.

Component III: Gaining the support from the school administrators, teachers, the school nurse and parents. In the process of implementing the program, the cooperation of school administrators, teachers, the school nurse and adolescents’ parents was crucial for success, since these stakeholders had important roles in this study as consultants and facilitators.

Step 7 Evaluating the outcomes and process of implementing the Internet-based program. The seventh step aimed to evaluate the outcomes and process of implementing the Internet-based program for promoting healthy eating behavior in early adolescents. To evaluate the outcomes, the knowledge, attitudes, eating behavior and nutritional status (weight for height) of adolescent participants were reassessed by the researcher in cooperation with the adolescent leaders. To evaluate the process of implementing the Internet-based program, participant observations were used to observe the atmosphere, the activities and responsive performance of adolescent participants while they were using the Internet-based program in the school. In addition, small group meetings of adolescent participants were conducted to describe their feelings and problems during implementing the program. Reflection on actions and problems concerned with program implementation was used and empowering was encouraged as well.

Step 8 Integrating the Internet-based program into the school system. The eighth step aimed to sustain and integrate the Internet-based program for promoting healthy eating behavior in the school system. The researcher, in cooperation with the adolescent leaders, organized a school meeting which included two school administrators, four teachers, the school nurse, ten representative early adolescents and three representative parents. In this school meeting, five adolescent leaders presented the details of the Internet-based program and effectiveness of the program by using a PowerPoint presentation. After presentation, open-ended questions were given to the group. These questions allowed the stakeholders to share their ideas about how to integrate and disseminate the Internet-based program in the school. As a result, the idea of using the Internet-based program for promoting healthy eating behavior and the suggested way to disseminate the program in the school by linking it to the school website, were accepted.

RESULTS

The findings of this study are presented in three parts: 1) unhealthy eating behavior issues in Thai early adolescents; 2) critical elements of the Internet-based program for promoting healthy eating in Thai early adolescents; and 3) outcomes of implementing the Internet-based program to promote healthy eating behavior in Thai early adolescents, using a participatory approach.
1) Unhealthy eating behavior issues in Thai early adolescents

The issues of unhealthy eating behavior among Thai early adolescents were gained from the stakeholders, including early adolescents, teachers, the school nurse and parents. The findings were analyzed and divided into four categories as presented in Figure 1: 1) eating preference foods without realizing their nutritional benefits or harmful effects, 2) eating as per the latest eating trends/fashion, 3) eating meals at irregular hours, and 4) eating foods lacking the five essential nutrient groups.

Figure 1. Illustration of unhealthy eating behavior issues in Thai early adolescents

2) Critical elements of the Internet-based program for promoting healthy eating in Thai early adolescents

After considering the suggestions of critical elements of the Internet-based program for promoting healthy eating behavior which emerged from the stakeholders, the adolescent leaders decided to plan developing the Internet-based program. They designed and developed the program’s components, which included the six critical elements as presented in Figure 2: 1) the contents promoting healthy eating behavior, 2) webboard discussions, 3) animations, 4) quiz exercises, 5) a game and 6) video clips. This Internet-based program was named by the adolescent leaders as the F-Club (Food Club).
3) The outcomes of implementing the Internet-based program to promote healthy eating behavior in Thai early adolescents using a participatory approach

The outcomes of implementing the Internet-based program could be categorized into four parts as follows:

3.1) Positive changes of the adolescent participants: These positive changes include knowledge of healthy eating behavior, attitudes towards healthy eating behavior, eating behavior and nutritional status.

**Improving knowledge of healthy eating behavior**

The results revealed that the scores of knowledge of food consumption immediately after implementing the program significantly increased compared to baseline in both the group of adolescent members ($Z = 6.64, p = .000$) and that of adolescent leaders ($Z = 4.19, p = .000$) according to the Wilcoxon signed-rank test as shown in Table 1.

Table 1. Comparison of the knowledge of food consumption between baseline and immediately after implementing the Internet-based program of the adolescent participants (N= 100).

<table>
<thead>
<tr>
<th>Adolescent participants</th>
<th>Time of evaluation</th>
<th>n</th>
<th>Mean rank</th>
<th>Sum of ranks</th>
<th>Z</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adolescent members</td>
<td>At baseline</td>
<td>73</td>
<td>14.50</td>
<td>14.50</td>
<td>6.64</td>
<td>.000*</td>
</tr>
<tr>
<td></td>
<td>At immediately after implementation</td>
<td>73</td>
<td>27.96</td>
<td>1696.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescent leaders</td>
<td>At baseline</td>
<td>27</td>
<td>0.00</td>
<td>0.00</td>
<td>4.19</td>
<td>.000*</td>
</tr>
<tr>
<td></td>
<td>At immediately after implementation</td>
<td>27</td>
<td>11.50</td>
<td>253.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant at the .05 level
Improving attitudes towards healthy eating behavior and having better eating behavior

The paired t-test was performed to analyze and compare the mean score of attitudes towards food consumption and the mean score of food consumption behavior between baseline and immediately after implementing the Internet-based program. The results showed that the mean score of attitudes towards food consumption \( t = 5.52, \ p < .000 \) and the mean score of eating behavior in adolescent members \( t = 2.02, \ p < .023 \) had significantly increased immediately after implementing the program, as compared with that at baseline. In addition, the mean score of attitudes towards food consumption \( t = 4.90, \ p < .000 \) and the mean score of eating behavior in adolescent leaders \( t = 4.53, \ p < .000 \) had significantly increased at immediately after implementing the program, as compared with that at baseline as well (see Table 2).

Table 2. Comparison of the attitudes towards food consumption and eating behavior between baseline and immediately after implementing the Internet-based program of the adolescent participants (N= 100).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Immediately after implementation</th>
<th>Mean difference</th>
<th>t</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Adolescent members (n=73)</td>
<td>72.70</td>
<td>8.11</td>
<td>77.71</td>
<td>8.09</td>
<td>5.01</td>
</tr>
<tr>
<td>Attitudes</td>
<td>88.93</td>
<td>8.45</td>
<td>90.71</td>
<td>8.53</td>
<td>5.52</td>
</tr>
<tr>
<td>Behavior</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescent leaders (n=27)</td>
<td>72.26</td>
<td>6.94</td>
<td>80.07</td>
<td>8.71</td>
<td>7.82</td>
</tr>
<tr>
<td>Attitudes</td>
<td>88.22</td>
<td>8.60</td>
<td>94.22</td>
<td>9.41</td>
<td>6.00</td>
</tr>
<tr>
<td>Behavior</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant at the .05 level

Improving nutritional status

After implementing the program, the researcher in collaboration with adolescent leaders, reassessed nutritional status (weight for height) of the adolescent participants (73 adolescent members and 27 adolescent leaders). Then the researcher analyzed the data of nutritional status at baseline and immediately after implementing the program by using descriptive statistics in terms of frequency and percentage. The results showed the improvement of adolescent participants’ nutritional status in both adolescent members and adolescent leaders as the increase in percentage of normal nutritional status and the decrease in percentage of unusual nutritional status including malnutrition, underweight, overweight, preobesity and obesity. In addition, the nutritional status of the adolescent participants between the baseline data and immediately after implementing the Internet-based program was also significantly different as indicated by the chi-square test (see Table 3).
Table 3. Nutritional status of the adolescent participants at baseline and immediately after implementing the Internet-based program (N=100).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline n (%)</th>
<th>Immediately after implementation n (%)</th>
<th>χ² test value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional status of adolescent members (n=73)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malnutrition</td>
<td>3 (4.11)</td>
<td>2 (2.74)</td>
<td>299.00</td>
<td>.000*</td>
</tr>
<tr>
<td>Underweight</td>
<td>7 (9.59)</td>
<td>6 (8.22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>41 (56.16)</td>
<td>46 (63.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>7 (9.59)</td>
<td>7 (9.59)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preobesity</td>
<td>6 (8.22)</td>
<td>4 (5.48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>9 (12.33)</td>
<td>8 (10.96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutritional status of adolescent leaders (n=27)</td>
<td></td>
<td></td>
<td>384.39</td>
<td>.000*</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>1 (3.70)</td>
<td>1 (3.70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>1 (3.70)</td>
<td>0 (0.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>17 (62.96)</td>
<td>20 (74.07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>4 (14.81)</td>
<td>3 (11.11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preobesity</td>
<td>1 (3.70)</td>
<td>1 (3.70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>3 (11.11)</td>
<td>2 (7.41)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant at the .05 level

3.2) Improving leadership competency of adolescent leaders

The researcher gained the information from the groups’ brainstorming and discussions with the adolescent leaders. They reflected the experiences gained from being adolescent leaders. The findings from this activity showed that they were impressed and proud of being adolescent leaders. They had learned how to work as a team from the research activities. Moreover, they had gained new knowledge about healthy eating behavior from the process of developing and implementing the program. Being adolescent leaders also enhanced their awareness of how to improve their eating behavior. This was a fruitful experience as well as providing useful knowledge for each of them that helped improve their leadership competency. They expressed their impressions from being adolescent leaders as shown by the following statements:

“We feel proud having participated in sharing views while developing this program and we have derived and gained a lot of experience in being adolescent leaders and having learned about the benefits of healthy eating behavior. The important part is – it is most enjoyable.”

“Being adolescent leaders in this study has given us good practice and better understanding of the teamwork spirit, especially unity in the group and the knowledge about consuming healthy and beneficial foods, and avoiding the unhealthy foods which are detrimental to our bodies and health. Thus, we have learned a lot about the real health–giving beneficial foods, in accordance with healthy eating principles.”
3.3) Satisfaction of using the Internet-based program

The opinions of adolescent participants towards the Internet-based program was assessed by using the Internet-based program’s satisfaction questionnaire, which has five scales ranging from ‘needs improvement’ up to ‘extremely good’. The results showed that adolescent members felt satisfied with the utility of the Internet-based program in respect of speed and ease, the attractiveness of the website, appropriateness of the screen’s design, the interest of the program and the usefulness of the website. In addition, qualitative data related to the satisfaction of adolescent participants towards the Internet-based program for promoting healthy eating behavior in early adolescents were obtained to confirm the results, by using open-ended questions to gain more of the adolescent participants’ feelings and opinions as shown by the following statements:

“It is a good informative program and makes us have positive attitudes towards the choosing of the right healthy and useful foods. Thus, it is a suitable Internet-based program which can be used anywhere and anytime, as today we use the Internet very often.”

“Animations, a game, webboard discussions and practices/quiz exercises are fun and encourage us to eat healthy foods. The contents are very interesting, enjoyable and appropriate for us.”

3.4) Integration the program into the school website

Sustainability of the findings was a final concern at the end of the study. After getting information from reflections, the stakeholders confirmed that the program was very useful and effective. The stakeholders gave ideas that this program should be disseminated to other Thai early adolescents to improve and promote their healthy eating behavior. The way to disseminate the program is to carry it over and link it to the school website and show it on first page, thus enabling adolescents and others to get into this program, in this way, easily disseminating the adolescents’ right eating behavior at a suitable length.

DISCUSSION AND CONCLUSION

The research findings indicate that using PAR to develop an innovative Internet-based program has the potential to promote healthy eating behavior among Thai early adolescents and enhances the leadership competency of adolescent leaders. It is observed from this study that the research participants’ involvement in all aspects of the PAR process was applied. The successful accomplishment and efficiency of this study may be attributable to the fact that it is in line with the concept of participatory action research (PAR) as a potentially-democratic process that is equitable and liberating, allowing participants to construct meaning in the process of group discussions (Koch et al., 2002), as also that the knowledge generated through PAR is no longer exclusively owned and disseminated by academia, but rather is shared by the community or group (Mill et al., 2001).
To be successful in implementing the program, teamwork should be encouraged and the involvement of adolescent leaders promoted. In this study, the adolescent leaders were the important stakeholders who played a major role in this program’s development and implementation. Obtaining the commitment of the adolescent leaders to participate in this study was also critical for the success in implementing the program, since the commitment of the participants ensured their whole-hearted devotion to its objectives. The involvement of the adolescent leaders in the study also ensured several aspects crucial to its sustainability and success, because such an approach provides the adolescents with a sense of ownership of the program. The involvement of adolescents in all steps of the research process is very crucial to its success. According to the study of Skinner et al., (1997), adolescents were integrally involved in all stages of the design, development, evaluation and dissemination of CyberIsle, a web-based program for changing adolescents’ smoking behavior. CyberIsle was designed and focused on health, personal and social issues identified by adolescents. It indicated that CyberIsle was a more relevant and enjoyable way of learning health information for adolescents than traditional health education classes. Bilal (2004) also recommends that researchers involve users in the design stage so that more effective interfaces that meet adolescents’ information needs and support their behaviors are developed.

Moreover, the cooperation of the adolescents’ parents was crucial for success in implementing the program as well. These parents supported the implementing of the program by devoting their time to send their children to school and take them home afterwards during the implementing phase, especially when the group meetings of adolescent leaders were held during the weekends. Therefore, the support from the stakeholder groups including parents and school administrators, as well as the participation of teachers and the school nurse, had made the implementation of the Internet-based program smoother towards its success. As shown from the statement of Gonzalez et al., (1991), action research should include several representatives from segments of the target community to guide or oversee the health promotion efforts, and a representative group might include: community residents and other influential people such as school personnel and the staff from community health centers.

As a result of implementing an Internet-based program study, the adolescent participants brought about positive changes in improving their knowledge of healthy eating behavior, improving attitudes towards healthy eating behavior, improving eating behavior, and improving nutritional status. These positive outcomes have shown that the Internet-based program was very efficient for promoting healthy eating behavior among Thai early adolescents. These results are congruent with the previous Internet-based studies which presented evidence of improving self-efficacy for healthy eating (Anderson et al., 2001; Long and Stevens, 2004; Suminski and Petosa, 2006), dietary knowledge (Long and Stevens, 2004; Suminski and Petosa, 2006), healthy eating behavior among adolescents (Anderson et al., 2001; Frenn et al., 2005; Williamson et al., 2005), and achieving reduction in body fat in girls (Williamson et al., 2005). The previous studies using the Internet have also shown
that the interventions available through the Internet and computer-assisted instructional programs are effective in promoting self-efficacy for healthy behaviors in adolescents and other groups (Anderson et al., 2001; Shegog et al., 2001; Long and Stevens, 2004). This indicates that the Internet can be highly beneficial for achieving health promotion in adolescents. It can increase data quality and save on costs in the long run, and it provides the opportunity to enhance the quality of adolescent health promotion (Mangunkusumo et al., 2006). The Internet is an innovative media used to promote the health of adolescents because they are typically the early adopters of new technologies (Skinner et al., 2003). The Internet is also a practical and effective way to deliver health information and interventions to adolescents (Borzekowski, 2006).

In conclusion, the research findings indicate that using PAR to develop an innovative Internet-based program has the potential to promote healthy eating behavior among Thai early adolescents and enhances the leadership competency of adolescent leaders.

ACKNOWLEDGEMENTS

This study was supported by the Commission on Higher Education of Thailand, the Thailand Nursing Council, and the Graduate School, Chiang Mai University, Thailand.

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none
Effects of Medium and High Discomfort Periods during Dry Environment on either Pathogens Causing Subclinical Mastitis or Antimicrobial Resistance of Environmental Streptococci and Coagulase-negative Staphylococci

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ABSTRACT

The objectives of this study were firstly to compare the prevalence of subclinical mastitis among pathogens between medium and high discomfort periods of the dry season in Chiang Mai, Thailand. Secondly, as the pathogens most frequently responsible for causing mastitis in Thailand, the resistant patterns of both environmental streptococci and coagulase-negative staphylococci (CNS) were also determined for both discomfort periods. Eighty small-holder dairy farms in Chiang Mai province, Thailand, were involved in the study. All clinically-healthy cows in the enrolled farms were used and tested for subclinical mastitis. Milk samples from subclinical mastitis cows were collected for bacteriological identification. Isolates from environmental streptococci and CNS were tested for their antimicrobial susceptibility. The periods were determined by levels of discomfort from heat and humidity, including December to February as medium discomfort period (MEDIUM) and November, March and April as high discomfort periods (HIGH). From a total of 691 cows, 40.1% of cows were positive to California mastitis tests (n=277). At udder level, most pathogens found in this study were minor pathogens, especially environmental streptococci (13.0%, 138 isolates) and CNS (9.9%, 105 isolates). The prevalence of mastitis with environmental streptococci and Staphylococcus aureus in MEDIUM were more than that in HIGH (P<0.05). In contrast, Enterobacteriaceae spp. in HIGH was higher than in the Medium discomfort period (P<0.05). The majority of the environmental streptococci isolates resisted to the antimicrobial agents (97.3%). No association was found between antimicrobial resistance against environmental streptococci and dry-discomfort environmental periods. For CNS, a total of 56% of CNS isolates were resistant to one or more antimicrobial drugs. During MEDIUM discomfort period, CNS was more resistant to cloxacillin and cephalaxin than...
during HIGH ($P<0.01$). In conclusion, there are some variations of pathogens causing mastitis and the antimicrobial resistant pattern of antimicrobial drugs against CNS mastitis between Medium and High discomfort periods.

Key words: Antimicrobial resistance, Coagulase-negative staphylococci, Dairy cattle, Dry discomfort environment, Environmental streptococci, Subclinical mastitis

INTRODUCTION

Mastitis is a costly disease in dairy industries (Bartlett et al., 1990), including clinical and subclinical forms. As the most prevalent type of mastitis, the subclinical form causes more damage in terms of overall economical losses. This is especially due to the fact that subclinical mastitis cows can be reservoirs of infection on most farms.

Bacterial intramammary infection is the most common cause of mastitis, and various bacterial species causing mastitis may be to blame, depending on time, geographic area, management and environmental factors. In general, mastitis occurrences were highest during the wet season in many countries such as Thailand (Rojstien et al., 2004), India (Joshi and Gokhale, 2006), Brazil (Costa et al., 1998) and Israel (Shpigel et al., 1998). In Europe, mastitis occurrences were more prevalent during the summer (Green et al., 2006; Olde Riekerink et al., 2007). The major pathogens associated with causing mastitis also differed among seasons (Waage et al., 1999; Osteras et al., 2006). Increases of mastitis occurrences in summer, however, were always associated with wet environments such as muddy areas (Suriyasathaporn et al., 2002). As a type of environmental streptococci, *Streptococcus uberis* contamination of paddocks and muddy places increased when wet conditions prevailed and when the cows’ grazing density was higher (Osteras et al., 2006). In addition, Zadok et al., (2005) showed that the proportion of fecal samples containing *Streptococcus uberis* was highest during the summer grazing season. Therefore, it is controversial whether or not hot environment without any wet environment (or dry season) is related to mastitis occurrences and the type of pathogens causing mastitis.

As a tropical country, Thailand has an average precipitation of a very high level during rainy season (above 100 mm) in comparison to European countries that have year-round averages at about 50 mm, even during their wet summer season (BBC weather, 2007). During dry season in Chiang Mai, Thailand, precipitation averages are very low and range between 0 to 30 mm (BBC Weather, 2007). This dry season can be separated into 2 periods, based on levels of discomfort from heat and humidity as MEDIUM (December-February) and HIGH (November, March and April) discomfort periods (BBC weather, 2007). Climate details during both discomfort periods of year 2007 are described in Table 1. Based on data from Table 1, maximum and minimum temperature averages in MEDIUM (29.7°C and 14.0°C, respectively) were lower than HIGH (33.3°C and 19.3°C, respectively). During the HIGH discomfort period, though not for MEDIUM, the
mean temperature-humidity-index (THI) is always above 78 (Suriyasathaporn et al., 2006), exceeding the critical THI point for lactating cows at 72 (Armstrong, 1994). Cows with heat stress were shown to have increased shedding of Enterobacteriaceae spp. such as E. coli (Edrington et al., 2004). Thus, the HIGH discomfort period might have differences with regard to the pathogens causing subclinical mastitis compared to the MEDIUM period. Therefore, the first goal of this study was to compare the prevalence of subclinical mastitis among pathogens between medium and high discomfort periods of the dry season in Chiang Mai, Thailand.

In Thailand, groups of pathogens such as CNS and environmental streptococci become dominant pathogens for subclinical mastitis (Ajariyakhajorn et al., 2003; Boonyayatra and Chaisri, 2004). Because antimicrobial drugs play an important role for the treatment and control of mastitis, therapy decisions are usually based on previous susceptibility information for the herd. The different susceptibility patterns among various mastitis pathogens have been widely reported, including groups of pathogens like CNS and environmental streptococci (Gentilini et al., 2002; Pikala et al., 2004; Mekonnen et al., 2005; Pol and Ruegg, 2007). However, information with regard to the seasonal differences in susceptibility or resistant patterns of both mastitis pathogens in the small-holder dairy farms in this area is limited. With regard to dry season variations, the second goal of this study was to determine the resistant patterns of antibacterial agents for both mastitis pathogens.

**MATERIALS AND METHODS**

**Animal and sample collection**

The study was performed during November 2004 to April 2005, using cows from small-holder dairy farms in Chiang Mai province, Thailand. All farms were members of their local dairy cooperatives, and farmers enrolled to participate into the study. All farms had approximately 5 to 15 milking cows housed in their tied-stall barns. For each farm, all clinically healthy lactating cows were tested, using the California Mastitis Test (CMT). The results were interpreted as follows: score 0 = no reaction; trace = slight slime that disappears with continued swirling; +1 = distinct slime but without gel formation; +2 = immediate formation of gel which moves as a mass during swirling; and +3 = gel develops a convex surface and adheres to the bottom of the paddle. A cow with a CMT score of ≥+1 at least one quarter was identified as a subclinical mastitis cow, and was included in the study. Milk samples from all quarters of the subclinical mastitis cows were separately collected with aseptic techniques in accordance with National Mastitis Council guidelines (NMC, 1999). The samples were kept in cool temperatures and transported to the laboratory immediately for bacterial identification.

**Bacterial identification**

Bacterial identification was performed according to the standard procedure described by National Mastitis Council’s guidelines (NMC, 1999). Ten microliters
An individual quarter milk sample was cultured on either a 5% bovine blood agar plate or a MacConkey agar plate. Plates were incubated at 37°C for 24-48 hours. Bacterial colonies were identified based on gross morphology, number of colonies and hemolytic pattern. Appropriate tests were performed on the isolated colonies to identify pathogens, including Gram staining and a catalase test to identify between streptococci and staphylococci. The hemolytic patterns and coagulase reaction with rabbit plasma were used to identify between \textit{S. aureus} and CNS. Esculin hydrolysis and CAMP reaction were used to differentiate \textit{S. agalactiae} and environmental streptococci. \textit{Arcanobacterium} spp. was identified by using culture characteristic on blood agar, motility and catalase reaction test. Gram-negative bacteria were identified as \textit{Enterobacteriaceae} spp., using culture morphology on MacConkey agar (Merck, Germany), lactose fermentation, motility and reaction in triple sugar iron. Other colony types were grouped as other microorganisms. The degrees of confidence in diagnosing an infection were classified as not significant, questionably significant, probably significant and highly significant, based on the National Mastitis Council’s guidelines (NMC, 1999). Samples that contained three or more bacterial species were considered to be contaminated. Isolates of either \textit{S. agalactiae} or \textit{S. aureus}, however, were always defined as intramammary infection (NMC, 1999).

**Susceptibility testing**

The highly significant isolates were tested for antibiotic susceptibility by the agar disk diffusion method in accordance with the standard procedure set forth by NMC guidelines (NMC, 1999). firstly, all isolates were checked for purity by subculturing on proper media. Three to five colonies of pure isolated pathogens were picked up and suspended in trypticase soy broth and incubated at 37°C for 2-8 hours to increase amounts of bacteria. The standard turbidity of bacterial suspension was adjusted to a turbidity equivalent to a 0.5 McFarland standard. The entire surface of agar plates was inoculated by using a sterile cotton swab. Commercially-prepared antimicrobial sensitivity discs, having the following antimicrobial agents and concentrations, were used: ampicillin (10 μg), cloxacillin (30 μg), cephalexin (30 μg), gentamicin (10 μg), erythromycin (10 μg), tetracycline (30 μg) and sulfathiazole (30 μg). Most of them were in the range of minimal inhibitory concentration (MIC) levels at which an isolate was considered susceptible according to Clinical and Laboratory Standards Institute guidelines (Pol and Ruegg, 2007). Discs were placed onto the agar surface and gently pressed to ensure contact. Plates were then incubated at 37°C for 24 hours. Subsequently, the diameter of the zone of inhibition around the disc was measured. The isolated microorganisms were categorized by susceptibility and resistance according to methods and criteria described by the National Committee for Clinical Laboratory Standards (NCCLS, 2002).

**Statistical analyses**

Contaminated milk samples were excluded from statistical analysis. Discomfort periods were defined by date during the collection of milk samples.
The periods were determined by levels of discomfort due to heat and humidity (BBC weather, 2007) including December to February as a medium discomfort period (MEDIUM) and November, March and April as a high discomfort period (HIGH). A summary of weather information on Chiang Mai is shown in Table 1. Frequencies of subclinical mastitis among pathogens and the resistant patterns were described as percentage. Effects of dry-discomfort periods on bacterial resistance were analyzed separately for each antimicrobial. The Fisher exact chi-square tests were used to evaluate the association of the dry-discomfort periods with either subclinical mastitis occurrence among pathogens or antimicrobial resistant pattern for both environmental streptococci and CNS. The significant levels were defined as P<0.05.

**Table 1.** Annual average of parameters on climate of Chiang Mai province, Thailand (BBC weather, 2007).

<table>
<thead>
<tr>
<th>Month</th>
<th>Average Sunlight (hours)</th>
<th>Temperature Average (°C)</th>
<th>Discomfort from heat and humidity</th>
<th>Relative humidity (%)</th>
<th>Average Precipitation (mm)</th>
<th>Wet Days (+0.25 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
<td>am</td>
<td>pm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan</td>
<td>9</td>
<td>13</td>
<td>29</td>
<td>Medium</td>
<td>96</td>
<td>52</td>
</tr>
<tr>
<td>Feb</td>
<td>9</td>
<td>14</td>
<td>32</td>
<td>Medium</td>
<td>93</td>
<td>44</td>
</tr>
<tr>
<td>March</td>
<td>9</td>
<td>17</td>
<td>34</td>
<td>High</td>
<td>88</td>
<td>40</td>
</tr>
<tr>
<td>April</td>
<td>9</td>
<td>22</td>
<td>36</td>
<td>High</td>
<td>88</td>
<td>49</td>
</tr>
<tr>
<td>May</td>
<td>8</td>
<td>23</td>
<td>34</td>
<td>Extreme</td>
<td>90</td>
<td>60</td>
</tr>
<tr>
<td>June</td>
<td>6</td>
<td>23</td>
<td>32</td>
<td>High</td>
<td>92</td>
<td>67</td>
</tr>
<tr>
<td>July</td>
<td>5</td>
<td>23</td>
<td>31</td>
<td>High</td>
<td>94</td>
<td>69</td>
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<tr>
<td>Aug</td>
<td>4</td>
<td>23</td>
<td>31</td>
<td>High</td>
<td>95</td>
<td>73</td>
</tr>
<tr>
<td>Sept</td>
<td>6</td>
<td>23</td>
<td>31</td>
<td>High</td>
<td>63</td>
<td>72</td>
</tr>
<tr>
<td>Oct</td>
<td>7</td>
<td>21</td>
<td>31</td>
<td>High</td>
<td>96</td>
<td>69</td>
</tr>
<tr>
<td>Nov</td>
<td>8</td>
<td>19</td>
<td>30</td>
<td>High</td>
<td>96</td>
<td>63</td>
</tr>
<tr>
<td>Dec</td>
<td>9</td>
<td>15</td>
<td>28</td>
<td>Medium</td>
<td>96</td>
<td>57</td>
</tr>
</tbody>
</table>

**RESULTS**

From a total of 691 cows, 40.1 % of cows were positive to CMT tests (n = 277). Because of collecting management and individual cow factors, only 1,085 milk samples from all subclinical mastitis cows were collected and used for bacterial identification. Approximately 1.9% of the samples (n = 21) were excluded because of bacterial contamination. From a total of 277 cows and 1,064 quarter samples, milk samples from 56.3% of cows (n = 180) and 27.8% of quarters (n = 291) had positive results on bacterial identification. At udder level, most pathogens found in this study were minor pathogens, especially environmental streptococci (13.0%, 138 isolates) and CNS (9.9%, 105 isolates). For major pathogens, *S. aureus* was found only 1.2% (13 isolates) and no *S. agalactiae* isolation was found. Percentages of mastitis pathogens isolated from quarter milk samples
divided by the discomfort periods are shown in Table 2. The prevalence of mastitis with environmental streptococci and S. aureus in MEDIUM were more than that in HIGH (P<0.05). In contrast, Enterobacteriaceae spp. in HIGH was higher than in the MEDIUM discomfort period (P<0.05).

**Table 2.** Percentages of mastitis pathogens isolated from quarter milk samples separated by the discomfort periodsa of Chiang Mai province, Thailand.

<table>
<thead>
<tr>
<th>Discomfort periodsa</th>
<th>Medium</th>
<th>High</th>
<th>χ²</th>
<th>P-valuec</th>
</tr>
</thead>
<tbody>
<tr>
<td>No growth</td>
<td>366</td>
<td>391</td>
<td>0.39</td>
<td>0.53</td>
</tr>
<tr>
<td>Environmental Streptococcus spp.</td>
<td>89</td>
<td>49</td>
<td>12.17</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>44</td>
<td>61</td>
<td>1.54</td>
<td>0.25</td>
</tr>
<tr>
<td>Arcanobacterium spp.</td>
<td>7</td>
<td>15</td>
<td>2.34</td>
<td>0.14</td>
</tr>
<tr>
<td>S. aureus</td>
<td>10</td>
<td>3</td>
<td>4.17</td>
<td>0.05</td>
</tr>
<tr>
<td>Enterobacteriaceae spp.</td>
<td>2</td>
<td>13</td>
<td>7.29</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>14</td>
<td>12.89</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total</td>
<td>518</td>
<td>546</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aThe periods were determined by levels of discomfort from heat and humidity as medium (Dec-Feb) (n=518) and high (Nov, March and April) (n=546) discomfort environments.

bA percentage of no growth sample was compared to all positive samples. Percentages of specified pathogen were compared to number of no growth samples.

cAssociations of pathogens with dry-discomfort periods were separately tested by Fisherís Exact test.

From a total of specified isolations, 81.9% and 70.5% for environmental streptococci (n = 113) and CNS (n=74), respectively, were successfully revived for susceptibility testing. The majority of the environmental streptococci isolates were resistant to the antimicrobial agents (97.3%). For environmental streptococci, percentages of resistance for seven antimicrobial agents in different periods are shown in Figure 1. Most antimicrobial drugs had high levels of resistance (ranges between 63% and 89%), except cephalxin and ampicillin, which had resistance levels of 15% and 27 to 34%, respectively. No association between antimicrobial susceptibilities versus environmental streptococci and dry-discomfort environmental periods was found.

For CNS, a total of 56% of CNS isolates were resistant to one or more antimicrobial drugs. Percentages of resistance for seven antimicrobial agents in different periods are shown in Figure 2. The average of resistance percentages for cloxacillin was highest, and the average resistance for cephalxin was lowest. During MEDIUM discomfort period, CNS resisted more to cloxacillin than HIGH (P<0.01). In addition, a resistance percentage of CNS to cephalxin during MEDIUM tended to be higher than during HIGH (P<0.1). No association between antimicrobial resistances and dry-discomfort periods among other antimicrobial drugs was found.
Figure 1. Percentages of antimicrobial resistance for Environmental Streptococcus spp. (n=113) separated by dry-discomfort periods. The periods were determined by levels of discomfort from heat and humidity as MEDIUM (Dec-Feb) (n=70) and HIGH (Nov, March and April) (n=43) discomfort environments.

Figure 2. Percentages of antimicrobial resistance for coagulase-negative staphylococci (n=74) separated by dry-discomfort periods. The periods were determined by levels of discomfort from heat and humidity as MEDIUM (Dec-Feb) (n=22) and HIGH (Nov, March and April) (n=52) discomfort environments. *, ** indicated association between dry-discomfort periods and antimicrobial susceptibilities at P<0.1 and P<0.05, respectively.
DISCUSSION

The climate of Thailand is influenced by the seasonal monsoon and the local topography. Most areas in Thailand including agricultural areas are recognized as tropical savannah. The tropical savannah climate is characterized by levels of precipitation during three distinct seasons: a hot dry season (March to May), a rainy season (June to October) and cool dry season (November to February). Therefore, the dry discomfort periods defined in this study were in the dry summer and cool seasons. From Table 1, the number of wet days (or precipitation higher than 0.25 mm) during this period are less than or equal to 5 days during the month, indicating that these periods are reasonably dry. Differences between MEDIUM and HIGH discomfort periods are determined by averages of maximum and minimum temperatures and relative humidity of both periods (Table 1). Although November has a low average temperature, the high humidity during the afternoon results in higher discomfort period than other months during the cool dry season in Thailand. To understand the effects of environment on animal production, THI was developed by the US Weather Bureau as a warm-weather discomfort index for evaluating conditions likely to result in livestock stress (Starr, 1981; Johnson, 1991) and is derived from measurements of air temperature and humidity. In this study, THI values during MEDIUM and HIGH using 3-month averages of temperatures and humidity, calculated by using equations from McDowell and colleagues (1979), were 69.3 and 75.9, respectively. This indicates that cows in HIGH were in heat stress status (Armstrong, 1994).

In this study, the prevalence of subclinical mastitis in dry season defined by the CMT test was 40.1%. The cow-prevalence of subclinical mastitis in tropical countries ranged between 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 ranging between 75.9% (Karimuribo et al., 2006) and 90.3% (Kivaria et al., 2004) in Tanzania. The huge variations among that prevalence might be related to the seasonal variation of the studies. In Chiang Mai, Thailand, Boonyayatra and Chaisri (2004) conducted studies using small-holder dairy farms and found that monthly subclinical mastitis prevalence ranged between 36.4 to 83.3%. By using bulk milk somatic cell count, Rojstien and colleagues (2004) suggested that subclinical mastitis was more severe during the rainy season. In comparison between the medium and high dry discomfort periods, no association was found with the prevalence of subclinical mastitis. In support of a previous study, heat stress did not reduce immune function capacity and did not relate to increased incidence of mastitis during the summer (Elvinger et al., 1991).

The highest prevalence of subclinical mastitis found in this study were environmental streptococci (13.0%, 138 isolates) and CNS (9.9%, 105 isolates), with low prevalence of subclinical mastitis from major pathogens such as S. aureus and S. agalactiae. This result was in agreement with previous reports in Thailand that both pathogens were the most frequent isolates (Ajariyakhajorn et al., 2003; Boonyayatra and Chaisri, 2004). A mastitis survey in Thailand showed that the most frequently found to cause mastitis during dry period was CNS (Leesirikul et al., 1994). In contrast, S. aureus was the most common cause of subclinical
mastitis in northeastern Thailand, Ethiopia and Kenya (Aiumlamai et al., 2000; Mekonnen et al., 2003; Shitandi and Kihumbu, 2004, respectively). However, CNS (Waage et al., 1999; Pitkälä et al., 2004; Rajala-Schulz et al., 2004) and environmental streptococci, especially for subclinical cases (Jayarao et al., 1999; Dingwell et al., 2004), have become the predominant pathogens for mastitis in many western countries. In Chiang Mai, the high emphasis on a mastitis control program by its cooperatives and university staffs could result in changing major mastitis pathogens in this area. In addition, the selected dry period in this study might cause the differences in pathogens causing mastitis.

From Table 2, the prevalence of mastitis with environmental streptococci and S. aureus in MEDIUM was more than that in HIGH. It is quite difficult to compare our results with previous studies because our environmental temperatures were relatively high and levels of precipitation were low (Table 1). Regardless of average precipitations, a study in Ohio where the average precipitation is the same year-round showed that the rate of environmental streptococcal intramammary infection (IMI) during a cow’s dry period and during lactation was greatest during the summer (Todhunter et al., 1995), when the average maximum and minimum temperatures range between 23 to 28°C and 11 to 16°C, respectively, which is comparable to the temperature range in the MEDIUM discomfort period in this study.

For S. aureus, many studies showed that warmer season did not reflect an increased prevalence of intramammary infection (IMI) or/and mastitis. For example, in Louisiana, the prevalence of S. aureus intramammary infection in breeding age heifers was much greater in fall than in summer (Fox et al., 1995). Data from Fox and Hancock (1989) showed an increased prevalence of S. aureus IMI during acute cold weather, indicating that season influenced the prevalence of IMI. In Thailand, a study using 4-year-old data showed that the overall rate of subclinical mastitis was highest during cooler months (Trisanarom et al., 1994). It is possible that high prevalence of subclinical mastitis in warmer climate might be caused by Streptococcus spp. and S. aureus. In contrast, we showed that Enterobacteriaceae spp. in HIGH was higher than in the MEDIUM discomfort period (P < 0.05). It is quite difficult to compare the prevalence here to other studies because of very high environmental temperatures in this study. However, it is possible that the increase of Enterobacteriaceae spp. during HIGH might be caused by increased shedding of Enterobacteriaceae spp. such as E. coli when cows were experiencing more heat stress (Edrington et al., 2004).

For CNS, the resistant patterns of all antimicrobial drugs were less problematic than environmental streptococci. The majority of the environmental streptococci isolates were resistant to the antimicrobial drugs (97.3%), compared to a lower percentage for CNS (56%) that were resistant to one or more antimicrobial drugs. Differences between resistance levels of these isolates may be caused by the difference of the MIC of antimicrobial for the isolates. Pol and Ruegg (2007) showed higher MIC levels of most antimicrobial versus environmental streptococci than were found in CNS. This resistance percentage of environmental streptococci was higher than those in previous reports (Busato et al., 2000; Erskine et al., 2002;
and Mekonnen et al., 2005). The highest resistance percentage of CNS was to \(\beta\)-lactams antibiotics, which is similar to many previous reports (Owens et al., 1997; Gentilini et al., 2002). Cloxacillin seemed to be the least useful antimicrobial for these isolates, as both isolates were determined to have a high resistance level to it.

In northern Thailand, a limited number of antimicrobial drug groups have been available for intramammary treatment of mastitis, the commercial products such as \(\beta\)-lactams (penicillin, cephalosporin groups, cloxacillin) and aminoglycosides (gentamicin). In addition, cloxacillin represents over 80% of the intramammary drugs available on the market. A wide variety of antimicrobial drugs have been used, often in an indiscriminate and inappropriate manner, impairing the solution of the problem or leading to its aggravation. Moreover, an important problem that arises from this kind of conduct is the increasing occurrence of microbial resistance (Susamo and Ocampo, 1992). Furthermore, the wide use of sulfa-trimethoprim, tetracycline and gentamicin to treat gastro-intestinal and other diseases in cattle has probably aided in developing resistance to these antimicrobial agents.

In this study, we found an association between the discomfort periods and the resistance of subclinical mastitis-causing CNS to the antimicrobial drugs cloxacillin and cephalaxin. Resistance levels during the cool season, MEDIUM, in Thailand were higher than in summer, HIGH, for both antimicrobial drugs (Figure 2). Two explanations that might be related to this finding include management factors and biological factors. For the management factors, it is possible that most cows in MEDIUM were just in the early postpartum period when most cows were receiving dry-cow therapy with antimicrobial drugs prior to performing the study. In northern Thailand, most cows are conceived during December to March (Punyapornwithaya et al., 2005). With regard to the biological factors, some studies found some seasonal variations on resistance to antimicrobial drugs. An example might be the huge seasonal association of the prevalence of penicillin-G resistance that was found in both \textit{S. aureus} and CNS (Osteras et al., 2006). Our finding supports this recent finding that CNS is seasonally resistant to cloxacillin and cephalaxin, both of which are \(\beta\)-lactams. A higher level of resistance was found more frequently during MEDIUM discomfort period. This finding was in accordance with the study of Osteras et al. (2006), who also found a higher proportional rate of penicillin resistance during the late indoor season. The reason for this is unknown; however, it was so characteristic that it will be important to investigate it in future studies. To our knowledge, there is, at present, no information in the available bovine mastitis literature on the seasonal occurrence of resistant pattern.

In conclusion, environmental streptococci and CNS were the most commonly isolated organisms responsible for subclinical mastitis in this area. During the dry periods (summer and cool-dry season), prevalence of mastitis with environmental streptococci and \textit{S. aureus} in the medium discomfort period, the cool-dry season, was higher than that in the high discomfort period, which refers to the dry part of the summer in Thailand. In contrast, the prevalence of \textit{Enterobacteriaceae} spp.
was higher in the high discomfort period. In Thailand, most of the environmental streptococci isolates were resistant to antimicrobial drugs (97.3%), while this was true for just over half (56%) of CNS isolates. Finally, we found an association between the discomfort periods and the resistance of subclinical mastitis-causing CNS to the antimicrobial drugs cloxacillin and cephalaxin. Resistance levels during the cool season, MEDIUM, in Thailand were higher than during the summer, HIGH, for both antimicrobial drugs.

ACKNOWLEDGEMENTS

First of all, this study was made possible by a financial support of the Royal Golden Jubilee (RGJ) Grant by the Thailand Research Fund (TRF). The authors would like to thank all farmers involved in the study for their kind cooperation. We also thank all staff of the Ruminant Clinic for their help as well as the staff of the milk quality laboratory, Faculty of Veterinary Medicine, Chiang Mai University for performing the bacteriological analyses of the samples. In addition, we would like to thank the Language Institute, Chiang Mai University, for their editorial support. Finally, we would like to thank Prof. Dr. Ynte H. Schukken, Cornell University, for his valuable comments.

REFERENCES


Maternal Participation in Caring for Newborns in an NICU

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ABSTRACT

The idea of maternal participation in caring for newborns in an NICU has been embraced into many hospitals nowadays. However, the caring practices used in NICU often intimidate the mother in performing her role. The purpose of this study is to understand and describe maternal participation in caring for newborns in an NICU. Non-participant observation and in-depth interview were used to collect the data in this qualitative research. 12 Thai mothers of newborns admitted to an NICU of a tertiary hospital in northern Thailand were recruited in this study as informants. Maternal participation is a continuous process, consisting of two phases; the initiation of participation and the best on-going actions for the sake of the baby. Moreover, mothers provided seventeen activities to the baby in both phases. These activities can be divided into two groups based on mothers’ intentions: activities intended to give warmth and encouragement to the babies and activities intended to ensure the babies’ safety.

Key words: Maternal participation, Caring for newborns in NICU, Critically-ill newborns, NICU

INTRODUCTION

The babies in an NICU have to be separated from their mothers to be under the care of medical staff for long periods (Whitfield, 2003) due to prematurity and abnormality which are major causes of their illnesses, requiring special treatments and NICU medical equipment (Ehrenkranz, 2006). Such departure since the babies’ birth not only interrupts the attachment process between the mothers and the babies (Schenk et al., 1992) but also brings suffering, stress and anxiety to the mothers (Holditch-Davis and Miles, 2000; Neu, 2004; Franck et al., 2005). As a result, the babies are often at a greater risk for cognitive and developmental problems, failure to thrive syndrome, parental abuse and neglect (Pillitteri, 1999; Hunter and Maunder, 2001; Aucott et al., 2002; Browne, 2003; Vorria et al., 2003; Ree, 2005). On the other hand, the mothers often depress and have problem in developing their role (Bell, 1992; Holditch-Davis and Miles, 2000; Hummel, 2003; Whitfield, 2003; Neu, 2004).
In order to eliminate negative effects of separation, hospitals facilitate mothers in building up a relationship with their babies by supporting the idea of maternal participation in NICU such as using a concept of family-centered care, development of NIDCAP program and using a kangaroo care (Cisneros Moore et al., 2003; Saunders et al., 2003; Malusky, 2005). However, the impact of the NICU environment, presence of highly trained professionals and the caring practices used in NICU often intimidate the mother in performing her role (Davis et al., 2003; Hall, 2005; Heermann et al., 2005), although she desires to provide both physical and spiritual supports to her baby (Schepp, 1992; Balling and McCubbin, 2001; Taya et al., 2002; Wigert et al., 2006). Moreover, some studies revealed that mothers feel their participation do not correspond to their need (Taya et al., 2002; Hall, 2005). This is due to factors such as inconsistency of policies, concern over infection and nurses’ attitude (Franck et al., 2002; Davis et al., 2003; Chia et al., 2006; Thomas, 2008).

However, in bringing Thai mothers into the care appropriately, apart from the knowledge from those studies nurses must have clear knowledge about maternal participation process and activities. Unfortunately, this crucial knowledge, which is documented, is rare, especially about newborns in the Thai culture. It is important to recognize that Thai and western concepts of participation are based on different socio-cultural contexts. Therefore, the results gained from previous studies may not be sufficient to describe maternal participation in Thailand. To clarify, Thai mothers usually believe that professionals know best about how to take care of the babies, so they tend to remain humble when decisions about the babies’ treatments have to be made (Pongjaturawit and Harrigan, 2003). In addition, Thai people from different regions have their own superstitious beliefs about possible causes and treatments of illnesses, as a result, Thai mothers often cope with the child illness problems in the ways corresponding to such beliefs, for example, longevity ceremony and changing the babies’ name (Jintrawet, 2005).

This qualitative study aimed to understand and describe characteristics of maternal participation in caring for newborns in an NICU in Thailand. The results of this study will provide nurses with critical insight and improve nurses’ understanding of mothers who have their babies in an NICU and their experiences. Therefore, nurses can improve their current and future care and practice to promote maternal participation with an aim to give more benefits to both mothers and babies.

**METHODOLOGY**

Informants included 12 Thai mothers of newborns admitted to the NICU of a university hospital in northern Thailand from August, 2007 to January, 2008. All participants volunteered. Inclusion criteria for informants were: (a) mothers of newborns admitted to the NICU for at least one week; (b) have visited the baby at least twice; and (c) ability to communicate in Thai. Once Faculty of Nursing and university hospital ethical approval was granted, mothers who matched the criteria were approached by a staff nurse of an NICU before they met the researcher. A
consent form was signed after the researcher informed mothers of the aims and details of the study.

Data were collected by using in-depth interviews and non-participant observations. After the written consent forms were returned, the researcher made an appointment with the participant for the first observation and interview. All participants were interviewed by the researcher three to four times, depending on the baby’s length of stay. Each interview took thirty to forty-five minutes in a private room or a comfortable area in the pediatric and obstetric ward. The first interview took place after the first observation while the last took place during last week of the baby’s admission in an NICU. Other interviews were done during the admission period of the baby. The researcher used an interview guide with general questions such as “Could you tell me about your experience in caring for your baby here?” or “Could you tell me what are you going to do when you come to see your baby?” During the time of the interview, the researcher probed more deeply on specific issues of participants’ activities such as “Could you tell me why you followed your baby to the operating room?”, “What did you do during your baby’s operation?.” The interviews were tape-recorded and conducted in Thai language. Data from non-participant observation served as a second source of data to provide additional data and to check for their reliability and validity. All participants were observed four to six times a week while they provided care for the babies both in and out of the NICU such as when they soothed the baby to sleep, when they escorted the baby to operating room or when they went to make a vow to the guardian spirits of hospital. After each interview and observation, the researcher recorded details on field notes for further analysis.

Content analysis described by Miller and Crabtree (1992) was used to analyze the data. First, all data were divided into three files: general information file, participation file and interpretation file. Second, the data from the participation file were coded along with collecting of the data. Interview data were transcribed verbatim in Thai language. Then the coding process was conducted from the transcripts. The data from non-participant observation and field notes were also used to help in the coding process. Coding process consisted of identifying unit, developing themes and categories. The developed themes were kept in the interpretation file. As the data were coded, themes and categories were changed until it was clear and constant enough to answer the questions of the study. Sampling continued until the point of saturation and no new data emerged.

For trustworthiness, techniques described by Lincoln and Guba (Lincoln and Guba, 1985 as cited in Holloway and Wheeler, 1996) including credibility, transferability, dependability and confirmability were used. Credibility was established by using methodological triangulation—both data from in-depth interviews and observation, prolonged involvement for 6 months and member check by participants after the data were analyzed. Thick description reflects the participants’ experiences in caring for the babies in an NICU to reach the potential transferability. Dependability was established by using tape recording, field notes and external check. All processes in this study were done systematically and each process can be audited. Therefore, confirmability was enhanced by the fact that
all of the processes and the results of this study were logical—every process can link together.

RESULTS

Research findings revealed that maternal participation was a continuous process composing of two phases; the initiation phase and the on-going phase, focusing on the actions for the best benefits of the baby. These two phases were not totally separated, as the actions/interactions found in phase 1 could be found in phase 2, particularly when the babies’ condition became worse or he/she needed an operation. Similarly, the actions/interactions related to phase 2 could be found in phase 1 if the mothers knew the baby was going to die (Fig. 1).

Phase 1: The initiation of participation

The initiation phase usually occurs during the first 2 weeks of the treatments in the NICU when the babies were critically ill. Maternal participation process in phase 1 was described as “an arrival at an unfamiliar world,” “facing difficulties and confusing feelings,” and “the desire to act for the babies.”

Arrival at an unfamiliar world

All mothers in this study had no previous experience of having a baby who was hospitalized in an NICU. Moreover, most of them were inexperienced in caring for a premature or abnormal baby. Therefore, everything mothers faced when their babies were admitted to an NICU was unfamiliar. Those things included the babies’ physical conditions and illnesses, medical treatments and equipment needed for the babies and spending time in hospital.

The babies’ physical conditions and illnesses terrified mothers. Some reported that they had bad experiences of seeing their babies stop breathing and they did not know what to do in such a situation. One stated, “It just happened, I was sitting there and talking to him, then his rate dropped so fast, both oxygen saturation and pulse rate. The oxygen saturation was reaching 30% and the pulse oximeter alarmed, and then his skin turned to blue. I was depressed. I mean, I lose hope every time they dropped. It was awful” (M03).

Moreover, severe illnesses caused the babies to depend on medical equipment such as ventilator and incubator. The sights of these instruments usually terrify mothers and make them misunderstand that they could not provide care to the babies. The majority of mothers admitted that medical equipment especially the ventilator scared them into touching or taking care of their babies because of the complicated handling required for the safety of the babies. Some also stated that they could not tolerate to be at the bedside. As well as an operation and a resuscitation which are the most frightening treatments that all mothers wished their babies did not experience as they believed them to be indicators of severity and loss of life. One said, “That day doctors told me his condition was badly off. They said he needed blood exchange and heart surgery and his lungs were bad. I dropped my breast-milk there and went outside. I did not dare to see him.
I thought I might lose him” (M07). These experiences are factors that prevent maternal participation. When mothers are unable to confront the truth, they fail to be fully informed of the babies’ problems and conditions provided by professionals.

Figure 1. Maternal participation in caring for newborns in an NICU.

Finally, all mothers have to change their daily lives during the admission of their babies. Mothers who stay at the hospital face problems such as sleeplessness and the discomfort of sharing a room with other mothers, as well as complying with the hospital regulations and policy such as vital sign monitoring and visiting hours for an unspecified period of time. On the other hand, mothers who stay at home had problems dealing with the inconvenience and exhaustion as one said “It was exhausting because I went back and forth 2-3 times a day and I could not get some sleep during the day like mothers who lived at hospital” (M03).

Moreover, all mothers had to cope with the rules and regulations of the NICU determined by professionals such as doctors and nurses. In adjusting themselves to these contexts, sometimes mothers felt more stressed and frustrated, especially when they were not allowed to be with the babies as one said “…in the daytime there are lots of staff, crowded in the room, so I had to leave my baby. It made me frustrated because doctors said if the baby could not sleep well, his weight would not increase but they disturbed him with noise” (M04).

**Facing difficulties and confusing feelings**

Mothers are usually overwhelmed by difficulties and confusing feelings while the babies are hospitalized in an NICU because of the babies’ illnesses and the specific requirements needed for the safety of the babies. Those feelings are “stress and anxiety,” “pity and fear of losing the babies”, and “confusion.”

Maternal stress and anxiety came from the fact that the babies’ illnesses and medical treatments were unknown to mothers. These feelings are intensified when the babies had to receive an operation or resuscitation which are believed
to be indicators of severity and the loss of life. Some confessed that the sight of the babies terrified them and scared them off. As one stated, “She looked so tired, grasped for breath. Her skin turned to blue. I started to cry right there. I could not bear it anymore, so I rushed out, I did not want my baby to be sad because of me. When I reached the elevator area, I punched it” (M05).

In addition, stress and anxiety of mother were increased by number of babies they got such as twins, triplets and quadruplets, as a result of double concern, responsibility and less time of caring for each baby. High level of stress caused them to provide inadequate breast-milk and also lost the opportunity to care for the babies as they could not communicate to people, both professionals and other mothers. They lost important information about the babies’ conditions and could not stop thinking that worse things could happen to the babies.

Second, all mothers felt pity and distressful when they experienced that the babies’ conditions got worse or the babies suffered from invasive procedures. They stated that they always cry and become subject of their fear of losing their babies—might not have a chance to see the babies alive again. Even a mother who was a nurse in PICU that had experiences in caring for a premature baby also reported that she had a similar fear like others: “the smallest I cared aged 24 weeks, weighed 800-900 grams. But she is my daughter, I was afraid; she was tiny and had low birth weight. I am afraid she may die” (M12). In addition, sometimes these feelings brought sadness and despair that led mothers to think they did not want the babies to be alive. One stated, “I told them to off the ET tube because I saw her in agony. She was tired, grasped for breath and her skin was blue. I did not want to see she suffer anymore” (M05).

Finally, mothers were filled with confusing feelings such as worry and guilt, especially when the babies received invasive procedures. Some stated that sometimes they felt the staff were not reliable or trustworthy and wondered what the staff did to their babies in the mother’s absence. Therefore, they barely left the NICU although it was time to eat or rest. Moreover, the majority of mothers felt guilty that they could not stay there to hold or help, and to protect the babies from pain. Guilt sometimes led mothers who blamed themselves and felt responsible for the babies’ illnesses—in case of criminal abortion and drug addicted mothers, to inflict pain by punishing themselves and thinking of committing suicide: “The first time I saw her, I wanted to jump from the roof. I was shocked and wanted to die if she dies” (M05).

The desire to act for the babies

All mothers love and worry about their babies’ safety, therefore no matter what happens. they desire to act for the babies which can be described as follows.

Being there with the babies in any situation

All mothers in this study had an intensive desire to be with their babies in an NICU all the time—to be nearby, to console, to care for and to help their baby to sleep; because they strongly believed that their love and encouragement are necessary for their babies as much as medical treatments. However, they also
realized that what they wanted was not possible and each visit brought them fear—that the babies’ conditions may worsen and they may lose their baby. Therefore, every time they came to visit their baby, they had to control their emotions and cope with fear in order to have a chance to care for the babies, to continue their visit, to spend as much time as possible at the bedside, to deliver breast milk, to observe their babies from outside the NICU while their babies received nursing care or treatment, and to follow their babies everywhere such as escorting their babies to an operating room. As one participant said, “I have to make up my mind every single day, to force myself against my feeling. I tell myself every time I brought him my breast milk that I must go and see him and if this time I couldn’t do it, I would try again and again. I kept doing this until I could see him without turning back” (M07).

Doing anything for the babies

All mothers tried to participate by doing anything to help the babies because they really believed the babies need them, although they could not cope with the unfamiliar environment they faced. Moreover, most activities they did are things that they had never done before. Some admitted that they kept telling themselves to practise until they could do it. The activities included visiting the babies everyday, delivering breast-milk, talking, touching, and caressing in order to give morale to the babies, encouraging the father to visit, maintaining breast-milk volume and seeking ways to save the babies by means of religious or supernatural beliefs such as making a vow to Joa Thee (the guardian spirit of hospital) and Phii Pu Ya (ancestor spirit). One said “My baby got ill because of karma (results of what one did in the past life). I do merit to help him, to relieve his sin by paying good merit to the one who he owes. So that bad things will end and he will recover” (M06).

Phase 2: The on-going phase focusing on the actions for the best benefits of the baby

This phase focuses on the best on-going actions for the sake of the babies. It usually comes after around 2 weeks of the initiation phase, depending on the babies’ physical conditions. In this phase, the majority of mothers felt relieved and had more actions for the babies as they tried to continue the actions started in phase 1. The participation can be described as facing reality, developing will-power with the babies and devotion to the babies.

Facing reality

During phase 2, mothers usually felt relieved and they realized that opening their mind was the only way to start their participation in caring for the babies. Therefore, mothers made themselves face reality by doing the following things.

Seeking the babies’ information

Mothers sought the babies’ information by asking and carefully listening to doctors and nurses although the information made them feel scared. Sometimes they also appreciated an opportunity to exchange their information about the babies to doctors and nurses because they believed that it would help their babies
to receive the best care and treatment. One stated, “I had to listen to whatever the doctors told me, I forced myself against my fear. Because I knew they would not call me if it was not important” (M05). Moreover, mothers often shared what they knew to each other such as the babies’ conditions and treatments, weight gain, activities they did to care for their babies and the means to maintain breast-milk volume because the information from mothers who have the babies with the same illnesses was a first-hand experience and this helped mothers to have a better understanding of the babies problems and treatments.

**Trying to familiarize with the babies’ conditions**
Mothers observed and memorized the babies’ symptoms such as grasping for breath and holding breath attentively, in order to familiarize themselves with the babies’ conditions. They believed that it would help them make the right decision whenever their babies needed help. Thus, they kept observing and memorizing until they were able to decide when they could provide the initial care for the babies and when to call for help. Then, they carefully observed nursing care such as how to change a diaper and how to hold the baby; to make sure that they could do it if necessary, because they wanted their babies to be safe. As one stated, “Such as when he holds his breath I watched him, If his belly moves but his chest doesn’t, it means he is holding his breath, or sometimes his chest still moves but the oxygen saturation continues to drop, that might be secretion obstruction and his skin will turn blue. In that case, I called a nurse” (M03).

**Providing care for the babies**
When mothers had confidence in themselves, they started to assist nurses and later give care by themselves under nurses’ approval as one stated “I came to care for her and helped the nurse when she passed urine or stool, or when tubes and wires slipped. I thought as a mother, at least, we should know about the tubes and wires our baby has to carry. Because whenever it slips, we can help our baby and inform the nurse, in case the nurse was not there” (M02). Moreover, they sought ways to provide physical comfort, to console their babies and help them to sleep such as holding hands, touching their head and singing.

**Developing will-power with the babies**
The majority of mothers described participation at this stage as developing will-power with their babies. Mothers developed will-power and provided mutual support to the babies because they strongly believe that their love and encouragement were necessary for their babies as much as medical treatments. Mothers developed will-power from the belief that their babies always give them mutual support by keeping themselves alive. Most mothers stated that the babies’ living is a promise from the babies that they are still fighting for their mother. By this belief, all mothers continued to provided mutual support with their babies even though they were filled with sadness and despair by talking and consoling the babies, helping the babies to sleep, being there with the babies, watching, following the babies everywhere they go, avoiding crying in front of the babies and giving amulets to the babies to protect them. However, mothers had their own ways of providing mutual support which vary from one to another, depending on
their belief and experiences.

**Devotion to the babies**

All mothers stated that the baby was the only reason for their enduring devotion such as maintaining volume of breast milk, adapting their daily life and continuing to provide care. They felt the babies needed and without the baby, they could not continue their participation till the babies are discharged as one said “… because of them, only them. If it was not for them, I believed I could not do all these things because I never did anything these much for anyone before. Everyday, I and my husband are so tired but when I thought my babies were waiting for me, I told myself to get up and be patient, even when I went to bed very late. I don’t know how to explain it but I would do anything for them” (M03).

Maternal devotions were influenced by one powerful feeling called “feeling happy about being a mother” that gradually developed in all mothers through the NICU experiences. This feeling was composed of two elements: connection between mothers and babies and the pride of caring for the babies. All mothers expressed that they believed in the idea of mother-baby bonding even in an NICU context because their babies kept showing the signs of connection such as waking up to wait for them every feeding time, crying at the time they were apart and turning their head following their mother’s voice. Thus, they tried their best to learn and train themselves to be able to provide a safe care with pride that they could give warmth along with encouragement by giving care by themselves as one stated “Although I was slow as a turtle, I took pride in doing it. I was so happy when I was able to do something by myself; I mean I could give warmth along with morale to her even when she was so sick. I helped nurses with most of things like taking a bath, changing the diaper and blowing wind with a handle-fan 3-4 hours straight. Only thing I did not do was hold her when nurse took her blood” (M05).

**Activities of maternal participation**

The results revealed that mothers did not want to replace the nurse in the NICU because they were aware of their limitations of knowledge and skills and concerned about the babies’ safety. However, mothers also believed their babies benefited most when they received warmth and morale along with nursing care. Therefore, mothers were willing to participate with nurses in caring for their babies. This study identifies 17 maternal participation activities that occurred in both phase 1 and 2. The frequency of activities in both phases varied according to the characteristics of the activities, for example, mothers observed and memorized the babies’ symptoms and nursing cares more often in phase 2 because these activities required mothers’ concentration, unfortunately, in phase 1 mothers were too overwhelmed by stress and unexpected situations to concentrate. Activities of maternal participation that occurred in both phase 1 and 2 could be divided into two groups on the basis of mothers’ intentions: activities intended to give warmth and encouragement to the babies and activities intended to assist nurses to ensure the babies safety.
Activities intended to give warmth and encouragement to the babies can be specified as the following:

1. Visiting the babies everyday
2. Being with the babies in any situation
3. Talking and caressing the babies
4. Helping the babies to sleep
5. Delivering breast-milk
6. Adapting their daily life
7. Avoiding crying in front of the babies
8. Encouraging father to visit the babies regularly

Activities intended to assist nurses to ensure the babies’ safety can be analyzed as consisting of the following:

1. Asking doctors and nurses for information about the babies’ conditions
2. Observing and memorizing the babies’ symptoms and nursing care
3. Exchanging information about babies with doctors and nurses
4. Sharing information with other mothers
5. Consoling the babies
6. Providing initial help to the babies when the babies show warning signs
7. Providing care and physical comfort to the babies
8. Maintaining volume of breast milk
9. Using other treatments related to their religious and supernatural beliefs to help the babies

**DISCUSSION**

The data analysis revealed that maternal participation in caring for newborns in an NICU is a continuous process, composing of two phases: the initiation of participation and the best on-going actions for the sake of the baby. In addition, all mothers in this study wanted to participate in caring for their babies since they knew that their babies were sick and their participation could be categorized into 17 activities inclusively of both participation phases. However, less participation was found in phase 1 compared to phase 2 because in the first phase, all mothers had to cope with various emotional crisis such as pity, fear of losing their babies, confusion and stress as a result of arrival at an unfamiliar world. These findings were similar to the results from previous studies that mothers who have their babies in the NICU suffered and worried about their babies (Melnyk and Gillis, 1998; Hummel, 2003; Whitfield, 2003) because they could not cope with the babies’ physical conditions, illnesses and medical treatments required.

However, all mothers strongly believed that their love and encouragement were very necessary for their babies as much as medical treatments in fighting with illness. Moreover, mothers stated that their babies fought for them by keeping alive. Therefore, they developed will-power and provided mutual support with their babies by trying their best to cope with difficulties and confusing feelings, and
participate by doing everything that, at that time, they could think was helpful for their babies such as visiting the babies everyday, delivering breast milk, avoiding crying in front of the babies and using other treatments related to their religious and supernatural beliefs to help their babies in phase 1. In phase 2, apart from continuing their activities, mothers got more involved in caring for their babies and endured their devotion until the babies could be discharged.

In addition, most mothers in this study kept helping each others such as giving mutual support and providing anything which was helpful for maintaining breast milk to each other, and then they formed an informal group-support to help new mothers by exchanging their knowledge about the babies’ illness and medical treatments, their experience in caring for their babies, and the way they cope with problems. This finding indicates that nurses can gain advantage in supporting mothers to participate by using group-support to find out more about mothers’ needs in order to respond to mothers more effectively.

Several activities found in this study such as visiting the babies everyday, exchanging babies’ information with doctors and nurses and providing care and physical comfort were similar to parents’ participation found in previous studies that divided participation into four aspects, namely, participation in routine care (Stull and Deatrick, 1986; Callery and Smith, 1991; Schepp, 1995), participation in nursing care (Stull and Deatrick, 1986; Schepp, 1995), participation in sharing information with professional (Stull and Deatrick, 1986; Schepp, 1995) and participation in decision making (Schepp, 1995; Neill, 1996).

However, other activities based on socio-cultural enlightening that Thai mothers used such as helping the babies to sleep, consoling the babies or using other treatment based on their religious and superstitious beliefs to help their babies, have not been mentioned in previous studies. For example, mothers intensively desired to do anything that could help their babies, therefore, they considered that using other treatments based on their religious and supernatural beliefs such as praying, making a vow to supernatural images like the guardian spirit of the hospital and ancestor ghost, doing merit, meditation, leaving an amulet at the babies bed and performing some ritual were needed too. These findings could prove that nurses have to be concerned about socio-cultural differences by continuing to assess mothers’ needs in participation—mothers’ beliefs, the way they want to participate and what they expect nurses to help with from time to time, in order to keep mothers participating in an appropriate way and avoid conflict between mothers and nurses. Nurses may block these activities which are important in the view of mothers by mistake—without knowing.

In addition, the beliefs that the babies’ illnesses were also related to Karma and good merit or supernatural power could help the babies were the results of socio-cultural enlightening which is passed to mothers from generation to generation (Chaisompan, 2002). Thus, nurses should include the socio-cultural context to ensure that misunderstandings between mothers and nurses, which can interrupt the participation, would not happen when mothers tried to use the alternative means to save their babies.
IMPLICATIONS

The results of this study provided knowledge and understanding related to maternal participation of Thai mothers in the care of newborns in the NICU. This knowledge will be useful for promoting maternal participation in an NICU as the mothers need to rely on nurses for support and approval in terms of knowledge and practice for the best results for the babies’ outcome. Most mothers had no experience in caring for newborns in NICU and listening for babies’ information, observing nursing care and sharing information with other mothers were the ways they used to begin their participation. Moreover, after they learned how to participate and come to care for the babies, they had to do it with nurses. Therefore, nurses should assess and provide all mothers with the babies’ information such as their physical conditions and illnesses, medical treatment and equipment they required and how mothers could participate appropriately to ensure that their participations were useful for the babies, in line with their wishes.

Moreover, nurses should assess and be concerned about maternal feelings as well as giving understanding and support to mothers during phase 1 because mothers are usually overwhelmed by stress and confused feelings, especially in the case of mothers who really felt guilty and tried to punish themselves, in order to help them to cope and to participate in an appropriate ways. Finally, nurses should be concerned about socio-cultural differences which have a connection to mothers’ beliefs when dealing with mothers in the NICU, in order to avoid any conflict between mothers and nurses that may occur when mothers try to use the alternative treatments to help their babies. In addition, providing group support for mothers will help nurses to keep in touch with mothers and to give mothers a chance to strengthen their ability in taking care of their babies by sharing the first hand experience and providing mutual support to each other.

REFERENCES


Lactic Acid Production by Coimmobilized Cells of *Lactococcus lactis* TISTR 1401 and *Lactobacillus casei* TISTR 1341 Using Whey as Substrate

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**ABSTRACT**

Lactic acid production from whey by batch fermentation of coimmobilized cells of *Lactococcus lactis* TISTR 1401 and *Lactobacillus casei* TISTR 1341 was investigated in order to decrease the manufacturing cost of lactic acid. The fermentation was conducted in a two liters fermentor at 37°C and pH 6.5 with an agitation rate of 100 rpm. The maximum lactic acid concentration was obtained with a value of 29.89 g.l⁻¹ and the coimmobilized cells had consistent potential to recycle two rounds of fermentation by producing 17.38 and 12.51 g.l⁻¹ lactic acid in 24 h, for each batch of the first and the second cycle, respectively, while lactic acid produced by free cells in mixed cultures of the two species of the bacteria was 16.63 g.l⁻¹ in 48 h. These results suggested that coimmobilized cell cultures were more effective than free cell mixed cultures in improving lactic acid production.

**Key words:** Whey, Lactic acid, Mixed cultures, Coimmobilized cell cultures

**INTRODUCTION**

Lactic acid is used as a biopreservative in food as lactic acid is effective in adding flavor and taste to food, controlling pH and inhibiting growth of microorganisms and germination of spores (Sachin et al., 2006). In addition, lactic acid is useful in biodegradable plastic production (Nabil et al., 2001). Lactic acid can be produced from chemical production or biological fermentation but the cost of chemical production is high and the product is difficult to be purified; therefore, biological fermentation by lactic acid bacteria (LAB) is made use of (Senthuran et al., 1999).

Synergistic effect of LAB has been reported recently regarding enhanced lactic acid production. KiBeom (2005) observed that mixed cultures of LAB might be more effective than single culture for improving lactic acid production. Moreover, immobilized cell technology can be established, leading to improved productivity (Sheng-Tsiung and Sheng-Tsiung, 1991).
Nowadays, research efforts are focused on looking for new and effective nutritional sources and new progressive fermentation techniques of both high substrate conversion and high production yields. Whey is the predominant substrate and usually contains about 5% lactose, 1% protein and 1% salts (Roukas and Kotzekidou, 1998), therefore, whey is used for lactic acid production because it is a relatively rich medium having high lactose and salt content, including some minerals (Pauli and Fitzpatrick, 2002; Fitzpatrick et al., 2003).

The aim of this study was to improve the production of lactic acid from biological fermentation of the mixed cultures of *L. lactis* TISTR 1401 and *L. casei* TISTR 1341 by using cell immobilization technique and using whey which is a by-product from the manufacturing of cheese and casein (Marshall, 1982) as the substrate.

**MATERIALS AND METHODS**

**Media**

Whey was received freshly from Minor Cheese Limited, a cheese plant in Bangkok. The whey was supplemented with 5 g.l\(^{-1}\) yeast extract, 10 g.l\(^{-1}\) peptone, 0.25 g.l\(^{-1}\) K\(_2\)HPO\(_4\), 0.03 g.l\(^{-1}\) MnSO\(_4\), 0.10 g.l\(^{-1}\) MgSO\(_4\) and 20 g.l\(^{-1}\) CaCO\(_3\) (Mostafa, 1995; Youseef et al. 2000). Its pH was adjusted to 6.5 before being sterilized at 121°C and 15 l.b/inch\(^2\) for 15 min.

**The Cultures**

*L. lactis* TISTR 1401 and *L. casei* TISTR 1341 used in the lactic acid fermentation were from TISTR Culture Collection, Bangkok Mircen, Thailand.

**Inoculum preparation**

Inocula of *L. lactis* TISTR 1401 and *L. casei* TISTR 1341, a homofermentative L(+)-Lactic acid producer, were propagated separately in 150 ml MRS broth in 250 ml Erlenmeyer flasks. The cultures were incubated at 37°C for 2 days and each species of the bacteria was used when the optical density (OD\(\text{660}\)) of the culture reached 0.5, with a total population of 7.50 x 10\(^6\) cfu ml\(^{-1}\). The cultures were used for inoculum 5, 7.5 and 10%.

**Immobilization of cells**

Each inoculum of mixed culture of the 5% *L. lactis* and 10% *L. casei* (Senthuran et al., 1999) was centrifuged at 3,000 g for 20 min and the spun broth was decanted. The pellet cells were resuspended in sterilized 0.85% NaCl solution and again centrifuged. After the NaCl solution was decanted, the pellet cells were mixed with sterilized 0.85% NaCl solution and sterilized 2% sodium alginate at a volumetric ratio of 10 : 3 : 2. The mixtures were then extruded by a peristaltic pump through the tube into 0.1 M CaCl\(_2\) solution to form beads. The distance from the end of the tube to the surface of the CaCl\(_2\) solution was 15 cm and the flow rate was 7 ml/min. The beads were suspended in CaCl\(_2\) solution at 4°C for 2 h and washed thoroughly twice with sterile distilled water before being used.
Fermentation conditions and cell recycling

The fermentation was performed in a two liters glass fermentor (B.Braun Biotech International Gmb H,D - 34212 Melhungen, Germany) with a working volume of 1.4 liters. The fermentor was sterilized at 121°C, 15 lb/inch² for 30 min. After cooling, the fermentor was inoculated with immobilized cells of L. lactis and L. casei. The fermentor was incubated at 37°C with an agitation rate of 100 rpm and the pH was maintained at 6.5 by automatic addition of sterile 5M NaOH. The sample was centrifuged at 10,000 g for 20 min and the supernatant was stored at 0°C for high performance liquid chromatography (HPLC) analysis.

For cells recycling, when the concentration of lactic acid was stabilized after first fermentation (Batch 1), the medium was then drained off from the fermentor and the fresh medium was added to the beads before a repeated batch (Batch 2) fermentation was started.

Assay Methods

The number of viable cells was determined by plate counting on MRS agar (A.O.A.C. 2000). The amount of lactose was detected by the protocol of Dubois et al. (1956). L(+)-Lactic acid concentration was measured by HPLC analysis. The HPLC system (SHIMADZU Co., Tokyo, Japan) was equipped with an Inertsil C8 - 3 column and was operated at room temperature using 20 mM KH₂PO₄ (pH 3) as the mobile phase. The flow rate was maintained at 1 ml /min. Lactic acid was detected by the UV detector at 210 nm. The concentrations of lactic acid were calculated by comparing the peak areas with the standard graph.

Data analyses

Data of the triplicate concentrations of lactic acid were used for statistical analyses by Duncan’s New Multiple Range test.

RESULTS AND DISCUSSION

Effects of inoculum sizes for lactic acid production by mixed cultures of free cells of L. lactis and L. casei in a two liters flask

The fermentation was studied with free cells pure cultures of 5% and 10% L. lactis and the maximum concentrations of lactic acid, 6.81 g.l⁻¹ and 8.48 g.l⁻¹, respectively, were obtained in 24 h when the cultures were incubated at 37°C in a stationary flask. The fermentations by free cells in pure cultures of 5% and 10% L. casei gave the maximum concentrations of lactic acid with the values of 7.36 g.l⁻¹ and 9.04 g.l⁻¹, respectively, in 84 h. It was found that 10% pure culture gave higher lactic acid than 5% pure culture while L. casei gave the highest lactic acid but needed much longer fermentation time.

For fermentation in mixed cultures of 5% each of L. lactis and L. casei, the maximum concentration of lactic acid obtained was 7.64 g.l⁻¹ within 60 h. It was found from this study that mixed cultures produced higher lactic acid than did the pure culture.

Fermentation in mixed cultures with 10% L. lactis and 10% L. casei, 5%
L. lactis and 10% L. casei, 10% L. lactis and 7.5% L. casei, 7.5% L. lactis and 7.5% L. casei gave the maximum concentration of lactic acid with the values of 10.71 g.l⁻¹, 11.40 g.l⁻¹, 10.70 g.l⁻¹ and 9.14 g.l⁻¹, in 60 h, respectively (Table 1). When lactose residues in mixed cultures and in pure cultures were compared, it was found that residued lactose in mixed cultures was less than that in the pure cultures, resulting in having higher lactic acid in the mixed cultures with the reason that more lactose was changed into lactic acid.

Table 1. Comparisons of various inoculum sizes of Lactococcus lactis and Lactobacillus casei for lactic acid production.

<table>
<thead>
<tr>
<th>Inoculum size</th>
<th>Fermentation time (h)</th>
<th>Concentration of lactic acid (g.l⁻¹)</th>
<th>Yield (g/g)</th>
<th>Productivity (g.l⁻¹.h)</th>
<th>Residued lactose (g.l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% L. lactis</td>
<td>24</td>
<td>6.81±0.163f</td>
<td>0.401±0.009</td>
<td>0.284±0.007</td>
<td>21</td>
</tr>
<tr>
<td>10% L. lactis</td>
<td>24</td>
<td>8.48 ± 0.245d</td>
<td>0.339±0.010</td>
<td>0.353±0.010</td>
<td>14</td>
</tr>
<tr>
<td>5% L. casei</td>
<td>84</td>
<td>7.36 ± 0.163e</td>
<td>0.526±0.012</td>
<td>0.088±0.012</td>
<td>20</td>
</tr>
<tr>
<td>10% L. casei</td>
<td>84</td>
<td>9.04 ± 0.163e</td>
<td>0.362±0.007</td>
<td>0.108±0.002</td>
<td>13</td>
</tr>
<tr>
<td>5% L. lactis and 5% L. casei</td>
<td>60</td>
<td>7.64±0.081e</td>
<td>0.294±0.003</td>
<td>0.127±0.012</td>
<td>11</td>
</tr>
<tr>
<td>5% L. lactis and 10% L. casei</td>
<td>60</td>
<td>11.40±0.326a</td>
<td>0.317±0.009</td>
<td>0.190±0.008</td>
<td>3</td>
</tr>
<tr>
<td>10% L. lactis and 5% L. casei</td>
<td>60</td>
<td>10.70±0.163b</td>
<td>0.324±0.005</td>
<td>0.178±0.002</td>
<td>7</td>
</tr>
<tr>
<td>10% L. lactis and 10% L. casei</td>
<td>60</td>
<td>10.71±0.161b</td>
<td>0.346±0.045</td>
<td>0.179±0.003</td>
<td>8</td>
</tr>
<tr>
<td>7.5% L. lactis and 7.5% L. casei</td>
<td>60</td>
<td>9.14±0.245c</td>
<td>0.315±0.009</td>
<td>0.152±0.004</td>
<td>10</td>
</tr>
</tbody>
</table>

*a,b,c,d,e,f means significantly different at 95% confidence level

Yun et al. (2003) studied the effects of carbon sources for lactic acid production of Enterococcus faecalis RYK 1 by using glucose, fructose, maltose, galactose, glycerol, xylose, whey and starch. It was found that using glucose, fructose and maltose produced 18.18 g.l⁻¹, 17.95 g.l⁻¹ and 16.80 g.l⁻¹ lactic acid, respectively while galactose, lactose, glycerol, xylose, whey and starch produced low concentrations of lactic acid in the values of 2.70 g.l⁻¹, 1.26 g.l⁻¹, 2.24 g.l⁻¹, 1.68 g.l⁻¹, 1.83 g.l⁻¹ and 1.19 g.l⁻¹, respectively.

Effects of agitation rate for lactic acid production by free cells in mixed cultures of L. lactis and L. casei in a two liters fermentor

Agitation rates at 0, 100 and 200 rpm gave the maximum Concentrations of lactic acid, i.e., 13.90 g.l⁻¹ in 60 h, 16.63 g.l⁻¹ in 48 h and 7.03 g.l⁻¹ in 48 h, respectively. At the 200 rpm agitation rate, the lowest amount of lactic acid was produced because the high agitation resulted in a higher shear rate and injured the cells. The fermentation of lactic acid by free cells in mixed cultures of 5% L. lactis and 10% L. casei in the fermentor was better than that in the flask.
because fermentation in the fermentor required shorter fermentation time and had higher concentration of lactic acid than that in the flask.

By using statistical analysis, it was found that the concentration of lactic acid produced at the agitation rate of 100 rpm was significantly higher (95% confidence level) than those at 0 and 200 rpm (Table 2).

**Table 2.** Comparisons of various agitation rates in a two liters fermentor by mixed cultures of 5% *Lactococcus lactis* and 10% *Lactobacillus casei* for lactic acid production.

<table>
<thead>
<tr>
<th>Agitation rate (rpm)</th>
<th>Fermentation time (h)</th>
<th>Concentration of lactic acid (g.l⁻¹)</th>
<th>Yield (g/g)</th>
<th>Productivity (g.l⁻¹.h)</th>
<th>Residued lactose (g.l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>60</td>
<td>13.90±0.24b</td>
<td>0.366±0.006</td>
<td>0.232±0.004</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>48</td>
<td>16.63±0.25a</td>
<td>0.414±0.020</td>
<td>0.348±0.017</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>48</td>
<td>7.03±0.21c</td>
<td>0.412±0.012</td>
<td>0.146±0.004</td>
<td>24</td>
</tr>
</tbody>
</table>

*a,b,c,d,e,f means significantly different at 95% confidence level*

**Effects of whey for lactic acid production by coimmobilized and free cells in mixed cultures of *L. lactis* and *L. casei* in a two liters fermentor**

Free cells fermentation by mixed cultures of 5% *L. lactis* and 10% *L. casei* in two liters fermentor produced 16.63 g.l⁻¹ lactic acid in 48 h. In an initial experiment, the concentration of lactose was increasing to be 40 g.l⁻¹ while the concentration of residued lactose was decreasing. The total amount of lactose, 40 g.l⁻¹, was produced in 80 h and a number of viable cells increased from 8.00x10⁶ cfu/ml to 2.00x10⁸ cfu/ml as shown in Figure 1.

The fermentation by coimmobilized cells gave the maximum concentration of lactic acid, 17.38 g.l⁻¹, in 24 h. Residued lactose decreased from 40 g.l⁻¹ to 0 g.l⁻¹ in 24 h as shown in Figure 2. The production of lactic acid by free cells when compared the fermentation between using free cell and coimmobilized cells, the shorter fermentation time and higher lactic acid production were found when using coimmobilized cells.

Chromopoulos et al., (2002) reported lactic acid fermentation by *L. casei* in free cells and in immobilized cells on gluten pellets. They were successful in immobilizing cells on gluten pellets, in fermenting glucose and sucrose in a shorter time (18 h), and in increasing the lactic acid production, 42 g.l⁻¹ and 41 g.l⁻¹, from glucose and sucrose, respectively.
Figure 1. Lactic acid production and number of viable cells in mixed cultures of 5% *Lactococcus lactis* and 10% *Lactobacillus casei* in a two liters fermentor.

Figure 2. Lactic acid production by coimmobilized cells of 5% *Lactococcus lactis* and 10% *Lactobacillus casei* in a two liters fermentor.
Data of the lactic acid used for statistical analyses between free and coimmobilized cells in mixed cultures are shown in Table 3. Concentrations and productivities of lactic acid of the two treatments were significantly different (95% confidence level) but the yields of lactic acid of the two treatments were not significantly different. Concentration, yield and productivity by the coimmobilized cells were higher than those by the free cells in mixed cultures.

**Table 3.** Comparisons of lactic acid production by free and coimmobilized cells in mixed cultures of 5% *Lactococcus lactis* and 10% *Lactobacillus casei*

<table>
<thead>
<tr>
<th>Form of cell</th>
<th>Fermentation time (h)</th>
<th>Concentration of lactic acid (g.l⁻¹)</th>
<th>Yield (g/g)</th>
<th>Productivity (g.l⁻¹.h)</th>
<th>Resided lactose (g.l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>free cells mixed cultures</td>
<td>48</td>
<td>16.63±0.25</td>
<td>0.414±0.020</td>
<td>0.348±0.017</td>
<td>0</td>
</tr>
<tr>
<td>coimmobilized cells</td>
<td>24</td>
<td>17.38±0.16</td>
<td>0.434±0.004</td>
<td>0.724±0.005</td>
<td>0</td>
</tr>
<tr>
<td>p-value</td>
<td>0.001*</td>
<td>0.921ns</td>
<td>0.001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns, p-value > 0.05 means not significantly different at 99% confidence level
*, p-value 0.0001 < p-value < 0.005 means significantly different at 95% confidence level

**Effects of fermentation by recycling coimmobilized cells of *L. lactis* and *L. casei* in a two liters fermentor**

The fermentation by coimmobilized cells of 5% *L. lactis* and 10% *L. casei* in Batch 1 produced maximum lactic acid, 17.38 g.l⁻¹, in 24 h while in Batch 2, the maximum lactic acid produced was 12.51 g.l⁻¹ in 24 h. However, after Batch 2, the coimmobilized cells could not be reused for the next cycle because when the drain medium from the fermentor was removed before pulting the new medium, it was found that the amount of the immobilized gels was decreased and therefore, fermentation could not occur in the next cycle with the reason that pH in the fermentor was controlled by NaOH which dissolved the gels. Figure 3 shows the concentration of lactic acid from Batch 1 and Batch 2. When concentrations of lactic acid from both batches were compared, Batch 1 gave higher lactic acid than Batch 2. The residued lactose of Batch 1 and Batch 2 was 0 g.l⁻¹ and 10 g.l⁻¹, respectively.

The comparisons of lactic acid production from fermentations in both batches are shown in Table 4. Concentrations and productivity of lactic acid from both batches were significantly different (95% confidence level) while the yield of lactic acid the two treatment were not significantly different.
Figure 3. The comparisons of lactic acid production by coimmobilized cells of Batch 1 and Batch 2 in a two liters fermentor.

Table 4. Comparisons of lactic acid production by immobilized cells of 5% *Lactococcus lactis* and 10% *Lactobacillus casei* in Batch 1 and Batch 2.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Fermentation time (h)</th>
<th>Concentration of lactic acid (g.l⁻¹)</th>
<th>Yield (g/g)</th>
<th>Productivity (g.l⁻¹.h)</th>
<th>Residued lactose (g.l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>17.38±0.16</td>
<td>0.434±0.004</td>
<td>0.724±0.005</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>12.51±0.41</td>
<td>0.403±0.013</td>
<td>0.521±0.017</td>
<td>10</td>
</tr>
<tr>
<td>p-value</td>
<td>0.001*</td>
<td>0.057**</td>
<td>0.001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns, p-value > 0.05 means not significantly different at 99% confidence level
*, p-value 0.0001 < p-value < 0.005 means significantly different at 95% confidence level

**CONCLUSION**

Lactic acid can be produced efficiently from whey by mixed cultures and coimmobilized cells of *L. lactis* TISTR 1401 and *L. casei* TISTR 1341. It was found in this experiment that the mixed culture of 5% *L. lactis* and 10% *L. casei* was the optimal Initial inoculum size for lactic acid production. Lactic acid production by the mixed culture in a two liters fermentor using the agitation rate at 100 rpm produced higher lactic acid than those produced at 0 and 200 rpm. For fermentation in a two liters fermentor, the coimmobilized cells produced higher lactic acid than the free cells of the mixed culture and reduced the fermentation time. Coimmobilized cells had consistent potential and could recycle only two rounds of fermentation. Batch 1 produced 17.38 g.l⁻¹ lactic acid while that produced by Batch 2 was 12.51 g.l⁻¹ and the fermentation times in both batches were 24 h.
REFERENCES


Roukas, T., and P. Kotzekidou. 1998. Lactic acid production from deproteinized whey by mixed cultures of free and coimmobilized *Lactobacillus casei* and *Lactococcus lactis* cells using fedbatch culture. Enzyme and Microbial Technology 22: 199-204.


Optimization of Gelatin Extraction from Thai Fish Panga 
(*Pangasius bocourti* Sauvage) Skin

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**ABSTRACT**

An investigation on optimal conditions for gelatin extraction from the Thai fish panga (*Pangasius bocourti* Sauvage) skin was performed by response surface methodology. A Box-Behnken design was applied to examine the effects of extraction temperature (40-70°C), pH (3.7-7.4) and extraction time (1-5 h) on gelatin yield, gel strength and gel colour. All regression models were significant (P≤0.01) and lack-of-fit of the models was insignificant, except for that of the gel strength. The Anderson-Darling normality test of the standardized residuals showed adequacy of all models. The optimal conditions for gelatin extraction were at 55°C, pH 4.55 for 1 h. The predicted responses were 20.22% gelatin yield, 506.55 g gel strength, 42.22 lightness (*L*), 3.56 chroma (*C*) and 43.35° hue angle (*h°*). The experimental responses of gelatin extracted at the optimal conditions were not significantly different (P>0.5) from the predicted value.

**Key words:** Gelatin, Thai fish panga, Response surface methodology, Physical properties

**INTRODUCTION**

Gelatin is a biopolymer obtained from partial hydrolysis of collagen. It has been used in many fields such as food, pharmaceutical, photographic and cosmetic industries. In food industry, it has been used as a gelling agent and an edible film. Gelatin can also promote healthy bones, joints and skin (Kasankala et al., 2007; Rahman et al., 2008).

Gelatin was previously extracted from bovine or swine skin or bones. However, since bovine spongiform encephalopathy (BSE) and foot-and-mouth disease had occurred, consumer became hesitant to eat food derived from these terrestrial animals. Fish are then an alternative source for gelatin production. Although it was reported that the bloom strength of fish gelatin was lower than that of bovine or swine gelatin, pretreatment of skin with saline or hydrogen peroxide solution could increase the bloom strength of fish gelatin (Giménez et al., 2005; Aewsiri et al., 2009).

The Thai fish panga (*Pangasius bocourti* Sauvage) is a new economic fish that has been promoted to be cultured in areas along the Mae Khong shore...
of Thailand. The fish is processed to frozen fillets for export to Europe and the USA. In the processing, many parts of the fish, such as skin and bones, are usually discarded (National Food Institute, 2006). However, the skin is composed of high amounts of collagen that can be converted to gelatin. Accordingly, the value is added to the skin by-products, and disposal problem is also diminished.

Our preliminary study found that pretreatment of fish skin with 0.8 M sodium chloride in 0.1 M sodium hydroxide solution resulted in increasing gelatin yield and gel strength compared with pretreatment with sodium hydroxide solution alone or hydrogen peroxide in sodium hydroxide solution. The principal objective of this study was to investigate an optimal condition for extraction of gelatin from the Thai fish panga skin pretreated with 0.8 M sodium chloride in 0.1 M sodium hydroxide solution, using acetic acid to adjust the pH. Gelatin yield, gel strength and gel colour were determined at various extraction temperatures (40-70°C), pH levels (3.7-7.4) and lengths of extraction time (1-5 h).

MATERIALS AND METHODS

Raw materials

The frozen Thai fish panga skin was obtained from a processing plant at Nakhonphanom province of Thailand and kept at -20°C prior to use. Proximate composition of the skin was 60.86% moisture, 35.83% crude protein, 2.19% crude lipid and 0.18% crude ash.

Reagents

Extraction chemicals included sodium hydroxide (Merck, Germany), sodium chloride (Union Science, Thailand) and glacial acetic acid (Labscan, Thailand). Analytical reagents included cupric sulfate 5-hydrate (J.T. Baker, USA), potassium sodium tartrate (Univar, Australia) and bovine serum albumin (Sigma-Aldrich, Canada).

Fish skin pretreatment

Fish skin was manually scraped off the flesh. The skin was then cut into the square dimension with the size of 1-2 cm. The fish skin was pretreated by stirring for 4 h in a solution of 0.8 M sodium chloride and 0.1 M sodium hydroxide at a skin-per-solution ratio of 1:20 (w/v). The solution was changed after 2 h of use. The pretreated skin was then rinsed 3 times with water before extraction with various concentrations of acetic acid solution.

Experimental design

The optimal condition for processing gelatin from the Thai fish Panga was determined by the response surface methodology. The Box-Behnken design was used to examine the effects of 3 independent variables—extraction temperature, pH and extraction time—on gelatin yield, gel strength and gel colour. The symbols and levels of independent variables are shown in Table 1. Five replicates at the central point of the designed model were used to estimate the pure error sum of squares.
Table 1. Experimental design range and levels of the independent variables for the production of the Thai fish panga skin gelatin.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Symbol</th>
<th>Range and levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coded value:</td>
<td></td>
<td>-1</td>
</tr>
<tr>
<td>Real value:</td>
<td></td>
<td>X₁</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td>X₂</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>X₃</td>
</tr>
</tbody>
</table>

Gelatin extraction

The pretreated skin was extracted by 17 treatments (Table 2). The pH of the extracting solution was adjusted to 3.70, 5.55 or 7.40 using glacial acetic acid. The pretreated skin was suspended in the extracting solution with the sample-per-solution ratio of 1:6 (w/v) (Kołodziejksa et al., 2008). Temperatures of the mixture were controlled at 40, 55 and 70°C using hot water bath. After extraction, the mixture was filtered through a piece of double-layer cheese cloth and then centrifuged at 2,000 g for 30 min to obtain gelatin solution as a supernatant. The protein content in the supernatant was determined. The gelatin solution was dried out overnight, using forced air oven at 50°C to obtain gelatin sheets with 13-14% moisture content. The dried gelatin sheets were measured for gel strength and colour.

Table 2. Experimental and predicted values of gelatin yields and gel strength responses of the gelatin extracted from the Thai fish panga.

<table>
<thead>
<tr>
<th>Standard order</th>
<th>Independent variables</th>
<th>Gelatin yield (%)</th>
<th>Gel strength (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature (°C)</td>
<td>pH</td>
<td>Time (h)</td>
</tr>
<tr>
<td>1</td>
<td>-1 (40)</td>
<td>-1 (3.70)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>2</td>
<td>+1 (70)</td>
<td>-1 (3.70)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>3</td>
<td>-1 (40)</td>
<td>+1 (7.40)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>4</td>
<td>+1 (70)</td>
<td>+1 (7.40)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>5</td>
<td>-1 (40)</td>
<td>0 (5.55)</td>
<td>-1 (1)</td>
</tr>
<tr>
<td>6</td>
<td>+1 (70)</td>
<td>0 (5.55)</td>
<td>-1 (1)</td>
</tr>
<tr>
<td>7</td>
<td>-1 (40)</td>
<td>0 (5.55)</td>
<td>+1 (5)</td>
</tr>
<tr>
<td>8</td>
<td>+1 (70)</td>
<td>0 (5.55)</td>
<td>+1 (5)</td>
</tr>
<tr>
<td>9</td>
<td>0 (55)</td>
<td>-1 (3.70)</td>
<td>-1 (1)</td>
</tr>
<tr>
<td>10</td>
<td>0 (55)</td>
<td>+1 (7.40)</td>
<td>-1 (1)</td>
</tr>
<tr>
<td>11</td>
<td>0 (55)</td>
<td>-1 (3.70)</td>
<td>+1 (5)</td>
</tr>
<tr>
<td>12</td>
<td>0 (55)</td>
<td>+1 (7.40)</td>
<td>+1 (5)</td>
</tr>
<tr>
<td>13</td>
<td>0 (55)</td>
<td>0 (5.55)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>14</td>
<td>0 (55)</td>
<td>0 (5.55)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>15</td>
<td>0 (55)</td>
<td>0 (5.55)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>16</td>
<td>0 (55)</td>
<td>0 (5.55)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>17</td>
<td>0 (55)</td>
<td>0 (5.55)</td>
<td>0 (3)</td>
</tr>
</tbody>
</table>

¹Numbers outside parentheses are coded values; numbers in parentheses are actual values.
Table 3. Experimental and predicted values of color responses of the gelatin extracted from the Thai fish panga.

<table>
<thead>
<tr>
<th>Standard order</th>
<th>Independent variables</th>
<th>Lightness (L*)</th>
<th>Chroma (C*)</th>
<th>Hue angle (h°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature (°C)</td>
<td>pH</td>
<td>Time (h)</td>
<td>Experimental value</td>
</tr>
<tr>
<td>1</td>
<td>-1 (40)</td>
<td>-1 (3.70)</td>
<td>0 (3)</td>
<td>45.62±0.72</td>
</tr>
<tr>
<td>2</td>
<td>+1 (70)</td>
<td>-1 (3.70)</td>
<td>0 (3)</td>
<td>42.00±1.50</td>
</tr>
<tr>
<td>3</td>
<td>-1 (40)</td>
<td>+1 (7.40)</td>
<td>0 (3)</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+1 (70)</td>
<td>+1 (7.40)</td>
<td>0 (3)</td>
<td>35.88±1.22</td>
</tr>
<tr>
<td>5</td>
<td>-1 (40)</td>
<td>0 (5.55)</td>
<td>-1 (1)</td>
<td>41.30±1.27</td>
</tr>
<tr>
<td>6</td>
<td>+1 (70)</td>
<td>0 (5.55)</td>
<td>-1 (1)</td>
<td>41.33±0.45</td>
</tr>
<tr>
<td>7</td>
<td>-1 (40)</td>
<td>0 (5.55)</td>
<td>+1 (5)</td>
<td>44.11±0.91</td>
</tr>
<tr>
<td>8</td>
<td>+1 (70)</td>
<td>0 (5.55)</td>
<td>+1 (5)</td>
<td>42.69±1.90</td>
</tr>
<tr>
<td>9</td>
<td>0 (55)</td>
<td>-1 (3.70)</td>
<td>-1 (1)</td>
<td>44.03±1.99</td>
</tr>
<tr>
<td>10</td>
<td>0 (55)</td>
<td>+1 (7.40)</td>
<td>-1 (1)</td>
<td>33.40±0.05</td>
</tr>
<tr>
<td>11</td>
<td>0 (55)</td>
<td>-1 (3.70)</td>
<td>+1 (5)</td>
<td>41.06±1.46</td>
</tr>
<tr>
<td>12</td>
<td>0 (55)</td>
<td>+1 (7.40)</td>
<td>+1 (5)</td>
<td>36.37±0.82</td>
</tr>
<tr>
<td>13</td>
<td>0 (55)</td>
<td>0 (5.55)</td>
<td>0 (3)</td>
<td>40.26±1.14</td>
</tr>
<tr>
<td>14</td>
<td>0 (55)</td>
<td>0 (5.55)</td>
<td>0 (3)</td>
<td>41.21±0.17</td>
</tr>
<tr>
<td>15</td>
<td>0 (55)</td>
<td>0 (5.55)</td>
<td>0 (3)</td>
<td>40.10±2.12</td>
</tr>
<tr>
<td>16</td>
<td>0 (55)</td>
<td>0 (5.55)</td>
<td>0 (3)</td>
<td>39.89±1.35</td>
</tr>
<tr>
<td>17</td>
<td>0 (55)</td>
<td>0 (5.55)</td>
<td>0 (3)</td>
<td>40.09±0.23</td>
</tr>
</tbody>
</table>

1Numbers outside parentheses are coded values; numbers in parentheses are actual values.

Gelatin yield determination

The protein content of the gelatin solution was determined by the Biuret method (Weaver and Daniel, 2003). In brief, 100 µl of the sample was mixed with 300 µl water and 1.6 ml Biuret reagent (0.15% copper sulfate and 0.6 sodium potassium tartrate in 3% sodium hydroxide solution). The solution was then kept for 30 min at room temperature before measuring the optical density at 550 nm, using bovine serum albumin as the standard. The gelatin yield was calculated as follows:

\[
\text{Yield (\%)} = \frac{\text{protein content in supernatant (g)}}{\text{weight of fish skin used (g)}} \times 100
\]

Gel strength determination

Gel strength was analyzed using to the method of Zhou and Regenstein (2004). Gelatin solution of 6.67% (w/w) was prepared by dissolving dried gelatin with distilled water and heated at 60±1°C for 30 min in a water bath. After that, the gelatin solution was filled in a cup (30 mm diameter × 15 mm height) and kept at 2±0.4°C for 16-18 h. The gel strength was measured by the texture analyzer (TA.XT Plus, Stable Micro System, England), using a 12.7 mm diameter plunger (P/0.5R probe), 0.5 mm/s compression rate and 4 mm penetration depth. The gel strength is a maximum force required in penetration.
Color measurement

The 6.67% gelatin solution was prepared as described above and measured for the color in L*C*h° scale, using Minolta Chroma Meter, CR300 model (Minolta, Japan).

Regression models

The response surface regression was analyzed, using the Design Expert software (Stat-Ease, Inc., USA). The following quadratic polynomial equation was a proposed regression model,

\[ Y_i = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{j=1}^{3-1} \sum_{j=1}^{3} \beta_{ij} X_i X_j \]

where \( Y_i \) were the dependent variables, \( \beta_0 \) was a constant, \( \beta_i \), \( \beta_{ii} \), \( \beta_{ij} \) were the regression coefficients and \( X_i, X_j \) were the independent variables. Some terms were excluded in this analysis to make the significant regression model with insignificant lack-of-fit, which has high correlation coefficient (R²). The optimal extraction condition that resulted in high gelatin yield, gel strength and lightness was obtained from the models.

The Anderson-Darling normality test was used to evaluate the adequacy of the model by plotting between the standardized residual (difference between the observed value and the predicted value divided by its standard deviation) of the dependent variables and their correspondence probabilities (Cho et al., 2005). The Minitab software (Minitab, Inc., State College, Pa, U.S.A.) was used in this analysis.

Model verification

Gelatin was extracted in triplicate using the obtained optimal conditions. Analysis of variance was carried out to test the difference between the experimental and the predicted optimal responses (Cho et al., 2005). A statistical analysis of this step was performed by the Minitab software.

RESULTS AND DISCUSSION

Response model

The experimental data are shown in Table 2. The physical properties of the gelatin extracted by treatment 3 were not evaluated because the gelatin yield was too low. The coefficients of independent variables, \( P \)-value and \( R^2 \) of the models, are shown in Table 4. All regression models were highly significant (\( P<0.01 \)) and the lack-of-fit was insignificant (\( P>0.5 \)), except for that of the gel strength.
Table 4. Coefficients of coded and uncoded independent variables with \( P \)-value and \( R^2 \) of models.

<table>
<thead>
<tr>
<th>Model details</th>
<th>Gelatin yield (%)</th>
<th>Gel strength (g)</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( L^* )</td>
<td>( C^* )</td>
<td>( h^\circ )</td>
</tr>
<tr>
<td>Coefficient of real value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( k )</td>
<td>-20.352</td>
<td>182.871</td>
<td>73.763</td>
</tr>
<tr>
<td>Temp</td>
<td>1.732</td>
<td>1.734</td>
<td>-1.013</td>
</tr>
<tr>
<td>pH</td>
<td>-7.553</td>
<td>138.071</td>
<td>1.493</td>
</tr>
<tr>
<td>Time</td>
<td>5.631</td>
<td>-16.604</td>
<td>-1.968</td>
</tr>
<tr>
<td>Temp(^2)</td>
<td>-0.019</td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td>pH(^2)</td>
<td>-0.518</td>
<td>-9.220</td>
<td>-0.590</td>
</tr>
<tr>
<td>Time(^2)</td>
<td>-0.772</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp \times pH</td>
<td>0.165</td>
<td>-0.747</td>
<td>0.032</td>
</tr>
<tr>
<td>Temp \times Time</td>
<td>-0.062</td>
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<td></td>
</tr>
<tr>
<td>pH \times Time</td>
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<td></td>
<td>0.401</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>( \beta_0 )</td>
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<td>482.721</td>
<td>40.500</td>
</tr>
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<td>( X_1 )</td>
<td>6.198</td>
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<td>-0.637</td>
</tr>
<tr>
<td>( X_2 )</td>
<td>-4.296</td>
<td>-9.904</td>
<td>-3.888</td>
</tr>
<tr>
<td>( X_3 )</td>
<td>2.286</td>
<td>-33.209</td>
<td>0.521</td>
</tr>
<tr>
<td>( X_1^2 )</td>
<td>-4.192</td>
<td></td>
<td>1.623</td>
</tr>
<tr>
<td>( X_2^2 )</td>
<td>-1.774</td>
<td>-31.557</td>
<td>-2.020</td>
</tr>
<tr>
<td>( X_3^2 )</td>
<td>-3.086</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( X_1X_2 )</td>
<td>4.578</td>
<td>-20.725</td>
<td>0.884</td>
</tr>
<tr>
<td>( X_1X_3 )</td>
<td>-1.852</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( X_2X_3 )</td>
<td>2.358</td>
<td></td>
<td>1.485</td>
</tr>
<tr>
<td>( P )-value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>&lt;0.0001</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>0.2618</td>
<td>0.0189</td>
<td>0.1065</td>
</tr>
<tr>
<td>Adjusted ( R^2 ) (%)</td>
<td>97.92</td>
<td>80.99</td>
<td>93.41</td>
</tr>
</tbody>
</table>

**Gelatin yield**

The response model for gelatin yield was

\[
Y_i = 21.400 + 6.198X_1 - 4.296X_2 + 2.286X_3 - 4.192X_1^2 - 1.774X_2^2 - 3.086X_3^2 \\
+ 4.578X_1X_2 - 1.852X_1X_3 + 2.358X_2X_3
\]

All terms were significant at 99% confidence level. The adjusted correlation coefficient of the model (\( R^2 \)) was 97.92%.

The gelatin yield increased with the rise of extraction temperature and the decrease of pH solution (Fig.1). This is because gelatin is well soluble in acid solution and the solubility is promoted by a high temperature (O’Neil et al., 2001). At a low temperature, collagen could be extracted and solubilized without altering its triple-helix configuration. At a high temperature, however, both hydrogen and covalent bonds are cleaved, the triple-helix configuration is destabilized and the helix-to-coil transition occurs (Montero and Gómez-Guillén, 2000). This phenomenon makes the solubilization of gelatin easier.
Figure 1. Response surface of gelatin yield (%) as a function of (A) extraction temperature and pH, (B) temperature and time, and (C) pH and time. (The third factor in each graph was fixed at the mid point.)
The gelatin yield also increased with the increase of extraction time. However, extraction for too long at low pH resulted in reduction of gelatin yield. The similar pattern was reported by Cho et al., (2006). According to the coefficient of model terms (Table 4), the extraction temperature was the main effect on the response, when compared with the pH level and extraction time.

**Gel strength**

The response model for gel strength was

\[ Y_2 = 482.721 - 36.169X_1 - 9.904X_2 - 33.209X_3 - 31.557X_2^2 - 20.725X_1X_2 \]

All terms were significant \((P \leq 0.05)\), except for the values of \(X_2\) and \(X_1X_2\), but they would be accounted for in the model to provide the high correlation coefficient \((80.99\%)\). The lack-of-fit \((P \leq 0.05)\) of the model indicated that the quadratic model may not be suitable for explaining the behavior of gel strength as a function of the three factors. This result agreed with the result of Yang et al., (2007), that the gel strength of gelatin extracted from the channel catfish skin could be predicted by neither the quadratic nor the linear model.

The gel strength decreased with the increase of temperature and extraction time (Fig. 2). Although gelatin can be extracted more easily at a higher temperature and with a longer treatment time, this severe condition would break the bonding and result in the release of free amino acid that causes reduction of gel strength (Cho et al., 2006). The maximum gel strength was observed at a pH level between 4.5 and 5.5, depending on the extraction temperature and time. From the results in Fig. 2A and Fig. 2C, extraction at pH lower than 4.5 would cause acid hydrolysis of gelatin molecules that results in the decrease of gel strength. Zhou and Regenstein (2005) reported that gelatin extracted from the Alaska pollock skin had the highest gel strength when extracted at pH 6, and that the gel strength decreased when the pH was lower or higher. The deviation of the results may come from the design points. This study was designed at pH 3.70, 5.55 and 7.40, while that of Zhou and Regenstein (2005) was designed at pH between approximately 3-9.
Figure 2. Response surface of gel strength (g) as a function of (A) extraction temperature and pH, (B) temperature and time, and (C) pH and time. (The third factor in each graph was fixed at the mid point.)
**Colour**

Gel colour is another factor that has been widely used to determine the physical quality of gelatin. The quadratic models of all colour responses were significant (P<0.01), with insignificant lack-of-fit (P>0.5). Table 4 presents the adjusted $R^2$ of the colour responses which were acceptable for the prediction of the responses. Lightness ($L^*$) of the gelatin was highly affected by pH (Table 4). Extraction of gelatin at higher pH caused gelatin to become darker (Fig. 3). Chroma ($C^*$) is used to describe the colour saturation of the objects. If the chroma value equal 0, the object color was white, grey or black depending on $L^*$. The more chroma value, the object became more colourful (Cruse, 2009). The chroma of the gelatin ranged between 2 to 5, which suggested that gel color was pale. The regression model of the chroma had many insignificant terms ($X_3$, $X_1X_2$ and $X_1X_3$) but these terms produced higher correlation coefficients, so these terms were accounted for in the model. According to the model, temperature and pH were the main effects on the chroma. Extraction at a high temperature and high pH caused the colour of the gelatin to have higher intensity (Fig 4). Temperature also proved to have a major influence on hue angle ($h^\circ$). Extraction at a high temperature caused gel color to change from pink to yellow (Fig. 5). Nevertheless, since the chroma value was quite low, variation of gel color or hue angle may not be visually observable. Thus, chroma and hue angle may not be deemed as the important factor to determine the quality of gelatin extracted by conditions used in this study.

**Normality test**

Normal probability plots of the standardized residuals are shown in Fig. 6 and Fig. 7. The standardized residuals greater than 2 and smaller than -2 are usually considered as large. The gelatin yield had two large residuals (Fig. 6A) while the gel strength, lightness and hue angle had one large residual (Fig. 6B, 7A and 7C, respectively), and chroma had no large residual (Fig 7B). According to the Anderson-Darling normality test, the standardized residuals of all responses had the normal distribution (P>0.5), indicating the adequacy of the models.

The distribution of gel strength’s residual was nearly significant (P=0.064). This result confirmed a significant model with lack-of-fit. Cho et al., (2005) also reported a similar pattern, that the quadratic model of gel strength had both significant model and lack-of-fit, although its residuals were distributed normally.

**Optimal condition**

In commercial production, the main purpose for extracting gelatin is to obtain gelatin with high yield. The gelatin should also have high gel strength and light color. Therefore, yield, gel strength, lightness and temperature were used in prediction of an optimal condition for the extraction of gelatin (Table 5). The optimal condition was extraction at 55°C for 1 h at pH 4.55, for which the predicted responses would be 20.22% gelatin yield, 506.55 g gel strength, 42.22 lightness ($L^*$), 3.56 chroma ($C^*$) and 43.35° hue angle ($h^\circ$).
Figure 3. Response surface of lightness ($L^*$) as a function of (A) extraction temperature and pH, (B) temperature and time, and (C) pH and time. (The third factor in each graph was fixed at the mid point.)
Figure 4. Response surface of chroma (C*) as a function of (A) extraction temperature and pH, (B) temperature and time, and (C) pH and time. (The third factor in each graph was fixed at the mid point.)
Figure 5. Response surface of hue angle (h°) as a function of (A) extraction temperature and pH, (B) temperature and time, and (C) pH and time. (The third factor in each graph was fixed at the mid point.)
Figure 6. Normal probability plots for error terms using standardized residuals of gelatin yield (A) and gel strength (B), based on the Anderson-Darling normality test.
Figure 7. Normal probability plots for error terms using standardized residuals of lightness (A), chroma (B) and hue angle (C), based on the Anderson-Darling normality test.
Table 5. Optimization parameters used in Design Expert software.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Goal</th>
<th>Lower</th>
<th>Upper</th>
<th>Weight</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>Minimum</td>
<td>40</td>
<td>70</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>Maximum</td>
<td>19</td>
<td>22.4</td>
<td>1</td>
<td>+++</td>
</tr>
<tr>
<td>Gel strength (g)</td>
<td>Maximum</td>
<td>500</td>
<td>587</td>
<td>1</td>
<td>+++</td>
</tr>
<tr>
<td>Lightness (L*)</td>
<td>Maximum</td>
<td>33.4</td>
<td>45.6</td>
<td>1</td>
<td>+</td>
</tr>
</tbody>
</table>

Cho et al., (2005) reported that an optimal condition for the extraction of gelatin from the yellowfin tuna skin was at 58.15°C for 4.72 h at pH of 6.0. The temperature reported by Cho et al., (2005) was close to this study, but their extraction time was much longer than that in this study. Liu et al., (2008) reported that an optimal condition for the extraction of gelatin from the channel catfish skin was extraction in 43.2°C water for 5.73 h at neutral pH. Kasankala et al., (2007) reported that the optimal conditions for gelatin extraction from the grass carp skin, pretreated for 24 h in 1.19% HCl solution, was at 52.61°C for 5.12 h. The discrepancy between this study and the previous studies may be mainly due to the difference in raw material and the pH used.

Model verification

The predicted and experimental responses of gelatin extracted at optimal conditions are shown in Table 6. The differences between the predicted and the experimental responses were insignificant ($P>0.5$), indicating that the regression models were suitable for the prediction of the studied responses.

Table 6. Experimental and predicted responses of the gelatin extracted at the optimal condition.

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Predicted value</th>
<th>Experimental value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin yield (%)</td>
<td>20.22</td>
<td>20.45±1.28</td>
</tr>
<tr>
<td>Gel strength (g)</td>
<td>506.55</td>
<td>508.22±22.05</td>
</tr>
<tr>
<td>Lightness (L*)</td>
<td>42.22</td>
<td>43.74±1.86</td>
</tr>
<tr>
<td>Chroma (C*)</td>
<td>3.56</td>
<td>3.31±0.61</td>
</tr>
<tr>
<td>Hue angle (h°)</td>
<td>43.35</td>
<td>42.68±4.71</td>
</tr>
</tbody>
</table>

CONCLUSION

The quadratic models as functions of extraction temperature, pH and time were suitable for the prediction of gelatin yield and gel colour. Although gel strength model had lack-of-fit, the results from normality test and model verification indicated that the gel strength model could be used to predict gel strength. The optimal condition for the extraction of gelatin from the skin of the Thai fish panga was at 55°C for 1 h at pH 4.55. The predicted responses from the optimal condition were 20.22% gelatin yield, 506.55 g gel strength, 42.22 lightness (L*), 3.56 chroma (C*) and 43.35° hue angle (h°). All values obtained from the experimental responses were in accordance with the predicted values.
REFERENCES


Distribution of Aquatic Macrophytes in the Coastal Area of Salimpur, Chittagong, Bangladesh


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ABSTRACT

This preliminary study was conducted to investigate the distribution pattern of the aquatic macrophytes in the inter-tidal coastal belt of Salimpur, Chittagong. During this study, 3 species of mangrove, i.e., Sonaratia apetala, Avicennia marina and Acanthus ilicifolius, 1 species of wild rice related to salt marsh grass, i.e., Porteresia coarctata, 3 species of macro-algae, i.e., Ulva intestinalis, Catenella nipae and Dictyota dichotoma and 1 species of poison lily Crinum defixum were identified from this coast. The dominant macrophyte was planted Sonaratia apetala, followed by Porteresia coarctata in the coast line of Salimpur. Considering from the ecological and economic view, especially Catenella nipae, could be an important living resource for cultivation and sea ranching in this area. Besides, the importance of these aquatic inter-tidal macrophytes for fishery resources and overall ecosystem processes should not be over looked in this coastal area.

Key words: Aquatic macrophytes, Salt marsh, Mangrove, Macro-algae, Salimpur, Chittagong

INTRODUCTION

Bangladesh is blessed with an extensive coastline of about 710 km, which is mostly covered by varieties of coastal living resources such as mangroves, salt marshes, sea grasses, macro and micro algae and fisheries (Pramanik, 1988). These coastal resources play a vital role in the life history development and food source of many coastal organisms. It is also well established that the coastal environment of Bangladesh is highly productive in terms of nutrient input from different sources, and promote the other living resources in the vicinity of the coastal environment. The diverse living resources in the coastal areas play an important role on the national economy as well as promote the socio-economic well-being of the coastal poor communities. Although these coastal resources contribute a vital role in the ecosystem and have a great significance in economic aspect, the study on the coastal plant resources and their usefulness are very limited. Till to date, except the studies by Das and Siddiqi (1985), no systematic investigation or inventory has been carried out on the diversity of the coastal macrophyte
resources together with their zonation pattern in the country. Few scientific data on macrophytes species are available for the coastal waters of Bangladesh and Indian Subcontinent (Islam, 1976; Salam and Khan, 1978, 1979; Islam and Aziz, 1987a, 1987b; Haider, 1993; SMRC 2000; Jagtap et al., 2002; Abu Hena et al., 2005; Jagtap and Nagle, 2007). Thus, any form of investigation on this coastal macrophytes resources and their environment condition can be considered to be important study in the country. Therefore, as a part of coastal study, this study deals with the diversity, distribution and zonation profile of the macrophytes growing in the inter-tidal coast line of Salimpur, Chittagong.

MATERIALS AND METHODS

Study Area Description

The study area is situated at the Salimpur coast, Chittagong and geographically located at 22° 15´ N latitude and 91° 49´ E longitude, and 15 km away from Chittagong port city. The study area is about ≥ 100 ha. The tidal range of this coast was about 2.43 m to 3.04 m throughout the year (Talukder, 2004). The muddy and sandy muddy alkaline soil substrate exits in the study area which is generally suitable for the growth of aquatic macrophytes.

Collection of Samples

This study was carried out during the months of April and May 2006. The zonation profile of the study area and distribution pattern of the macrophytes were observed physically by placing three transects perpendicular to the shore (English et al., 1994). The different types of macrophytes specimens were collected manually by hand or using a knife during the low tide. All samples were collected in the pre labeled plastic bag while macro algae were collected in the plastic pots containing 5% formalin. All the collected samples were brought back to the Laboratory of Estuarine, Coastal and Aquaculture Research (LECAR), Institute of Marine Sciences and Fisheries, University of Chittagong and washed under tap water. The identification of the specimens was done following the literature described by Singh and Garge (1993) for mangroves, Lewmanomont and Ogawa (1995) and Islam (1976) for macro-algae, followed by Chapman (1977) and Flowers et al., (1990) for salt marsh.

RESULTS AND DISCUSSION

The species list of aquatic macrophytes found in the Salimpur inter-tidal coast and their major ecological functions is given in Table 1. A tentative zonation profile of the study area of Salimpur is presented in Figure 1. During this study, three species of mangrove, i.e., Sonaratia apetala, Avicennia marina and Acanthus ilicifolius, one species of wild rice salt marsh, i.e., Porteresia coarctata, three species of macro-algae, i.e., Ulva intestinalis, Catenella nipae and Dictyota dichotoma and one species of poison lily Crinum defixum were identified from this coast.
Table 1. Coastal aquatic macrophytes and their ecological functions in Salimpur, Chittagong.

<table>
<thead>
<tr>
<th>Group</th>
<th>Species</th>
<th>Status and ecological function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mangroves</td>
<td><em>Sonarata apetala</em> <em>Avicennia marina</em> and <em>Acanthus ilicifolius</em></td>
<td>Planted and growing naturally; fishery importance; ecosystem stability; nutrient input and habitat for coastal fishes and birds, and coastal environment.</td>
</tr>
<tr>
<td>Macro algae</td>
<td><em>Ulva intestinalis</em> <em>Catenella nipae</em> and <em>Dictyota dichotoma</em></td>
<td>Primary producer; direct food source of many animals including human; provide shelters for number of marine and coastal species.</td>
</tr>
<tr>
<td>Salt marsh</td>
<td><em>Porteresia coarctata</em></td>
<td>Strong dilution and stabilization of pollutants from terrestrial run off and tidal waters flow through marshes; nutrient supply that are as important part of marine food chain; spawning and nursery area; refuge habitat for many fish and shellfish species; nesting and feeding areas of shore birds and wild life.</td>
</tr>
<tr>
<td>Other aquatic plants (poison lily)</td>
<td><em>Crinum defixum</em></td>
<td>Coastal stabilizer and habitat of macro and microorganisms.</td>
</tr>
</tbody>
</table>

Figure 1. Schematic zonation pattern of macrophytes at Salimpur coast, Chittagong (based on three transects).

The most of the mangrove species were planted *S. apetala* in the intertidal area of Salimpur coast under the green belt project of Bangladesh (Mahmood, 1986; 1995), which are colonized by macro-algae and other coastal plants naturally through succession. The mangrove *S. apetala* was found as four-species association with the salt marsh (*P. coarctata*), macro-algae (*U. intestinalis, C. nipae* and *D. dichotoma*) and *A. ilicifolius/C. defixum* in this study area. Infrequently, *A. ilicifolius* and *C. defixum* were found as patchy form in this inter-tidal coastal area. This type of mangrove exists in other coastal area of Bangladesh (Zafar, 1992). The almost of the macro-algae grow on the mangrove roots in the coast of Salimpur, especially *C. nipae*. Other types of macro-algae usually creep with segmented thallus associate with decomposed mangrove twigs and leaves.
acting as growing substrate. However, some studies suggested that the prospect of macro-algae culture in Bangladesh is very rich and potential which could support to the national economy (Zafar, 2004).

The wild rice P. Coarctata, relative salt marsh grass, dominates the regularly-flooded low marsh in the study area of Salimpur. Similarly, salt marsh P. coarctata was found growing in the inter-tidal brackish water in river mudflat system (Jagtap et al., 2006), and estuaries and marine environment elsewhere (Table 2). Salt marsh grass is the most abundant salt-tolerant plant in most of the estuarine environment of Bangladesh and responsible for much of the marsh productivity. The salt marsh P. coarctata was found as a mono-specific association and sometime it grows as two-species association with A. ilicifolius, macro-algae (U. intestinalis, C. nipae and D. dichotoma) or mangrove (S. apetala and A. marina). Altogether, there are 5 genera (P. coarctata, Imperata cylindrica, Eriochloa procera, Myriostachya wightiana and Phragmites karka) of salt marsh grass in the coastal and estuarine area of Bangladesh which also grow in the South Asian and South East Asian subtropical and tropical coasts (Das and Siddiqi, 1985; Abu Hena et al., 2007b). Among 5 species of salt marsh grasses, P. coarctata is dominat in different geographical regions, i.e., Eastern and Western coasts of India, coast of Sri Lanka and coast of Karachi, Pakistan (Latha et al., 2004). It has extensive rhizome, root, stem and leaf systems which are almost similar to those seen in the species of genus Spartina spp. found in temperate salt marsh habitat, i.e., Central American coasts (Caribbean-Eastern-Pacific), South American coasts, North American coasts and also harboring in the Western Indo-Pacific coasts (Hitchcock, 1951; Alderson and Sharp, 1994). The salt marsh grass Porteresia’s successful adaptations enable it to live where only few other plants could survive. It has narrow and tube-shaped stem, tough leaf blades and special glands that secrete excess salt, making it ideal to withstand the high heat and daily exposure to sea water. Some herbivores feed directly on salt marsh, especially cattle, and a substantial fraction of plant carbon enters into the coastal and estuarine food web through the microbial process of litter and particulate organic detritus (Abu Hena et al., 2007a and 2007b). Salt marsh meadows physically filter suspended sediments from the water, help reduce wave and current energy and stabilize bottom sediments of the coastal area (Day et al., 1989). Therefore, this habitat is among the most productive ecosystem in the world in term of the quantity of vegetation produced annually per unit area (Gosselink et al., 1974; Day et al., 1989). The high primary production rates of salt marsh are closely linked to the high production rates of associated fisheries in the study area of Salimpur coast, Chittagong.

ACKNOWLEDGEMENTS

The authors are grateful to University of Chittagong for their financial support partially to carry out the present work. The authors also want to express their gratitude to the Director, Institute of Marine Sciences and Fisheries, University of Chittagong for providing the necessary facilities pertaining to the work.
Table 2. Location and habitat description of salt marsh grass *Porteresia coarctata*.

<table>
<thead>
<tr>
<th>Location</th>
<th>Habitat description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prentice and Chukdar Islands,</td>
<td>Coastal mudflat and marine environment growing with mangrove ecosystem in mono specific condition and tow species association</td>
<td>Misra et al. (1998)</td>
</tr>
<tr>
<td>India</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goa coast, India</td>
<td>River mudflat with mangroves and coastal area growing in mono specific form with patches</td>
<td>Jagtap et al. (2006)</td>
</tr>
<tr>
<td>Cox’s Bazar, Bangladesh</td>
<td>Estuarine intertidal zone and river bank with seagrass (H. beccarii), mangroves (Avicennia alba, A. marina and Acanthus ilicifolius) and macro algae (Ulva intestinalis) and salt marsh (Imperata cylindrica)</td>
<td>Abu Hena et al. (2007a; 2007b)</td>
</tr>
<tr>
<td>Salimpur, Chittagong</td>
<td>Coastal intertidal zone with mangroves (Avicennia marina, Sonaratia apetala and Acanthus ilicifolius) and macro algae (Ulva intestinalis, Catenella nipae and Dictyota dichotoma) and poison lily (Crinum defixum)</td>
<td>Present study</td>
</tr>
</tbody>
</table>

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Effect of Accelerated Aging Treatments on Aroma Quality and Major Volatile Components of Thai Jasmine Rice

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ABSTRACT

The effect of accelerated aging (AA) treatments on aroma quality and major volatile components of freshly-harvested Thai jasmine rice cv. Khao Dawk Mali 105 was investigated. Freshly-harvested milled rice were exposed to three AA conditions which were 100°C for 100 min, 110°C for 45 min and 120°C for 25 min, and then their aroma quality was evaluated. The aroma quality was assessed on the basis of the quantity of aroma-impact compound, 2-acetyl-1-pyrroline (2AP), and an off-odor compound, n-hexanal, using GC-FID. Other volatile components were also analyzed by GC-MS. Results revealed that the quantity of 2AP and n-hexanal decreased in AA samples. However, the AA rice had better aroma quality when compared with that of 3-month naturally-aged rice. Analysis of rice volatile components indicated that the AA treatments did not affect the volatile constituents that make up for odor character of this aromatic rice. Thirteen identified compounds: n-hexanal, n-heptanal, 2-acetyl-1-pyrroline, benzaldehyde, 1-octen-3-ol, 2-pentylfuran, 1-octanol, n-nonanal, n-dodecane, n-decanal, n-tridecane, (E)-2-tetradecene and n-tetradecane, found in freshly-harvested rice, were all present in the AA samples with no addition of new volatiles. From these results, it can be concluded that the AA technique can bring freshly-harvested rice cv. KDML 105 to advanced stage of aging while still maintaining its high aroma quality.

Key words: Aromatic rice, Accelerated aging, 2-acetyl-1-pyrroline, n-hexanal, Volatile components
INTRODUCTION

Aging can improve some of cooking and eating properties of rice that is preferred by Asian consumers. However, aging process takes time and at the same time can reduce some desirable characteristics including aroma of fragrant rice. To shorten time of this conventional aging, a technique called accelerated aging (AA) had been proposed. Accelerated aging of freshly-harvested paddy, using wet or dry heat treatments with suitable grain moisture content had been studied and reported to improve cooking quality of rice which resembled to those of naturally-aged rice (Gujral and Kumar, 2003; Soponronnarit et al., 2008). However, the consequence of this accelerated aging technique on aroma characteristic of rice has not yet been investigated and verified. Such AA practice on freshly-harvested paddy could have impacts on the aroma quality and volatile profile, and could probably change the typical aroma characteristic of rice. This was due to the diffusion of husk and bran components into endosperm of rice during moistening step and the relatively high temperature employed in aging of the moist paddy, as occurred in parboiled rice (Lamberts et al., 2006).

Volatile compounds that provide aroma characteristic of fragrant rice had been studied by a number of researchers. A relatively large number of compounds from uncooked (Mahatheeranont et al., 2001) and cooked (Buttery et al., 1983a, 1983b, and 1986; Paule and Powers, 1989; Widjaja et al., 1996a; Yang et al., 2008) aromatic rice had been identified. Research results also indicated that aroma of the rice was composed of a mixture of numbers of odor-active compounds and these compounds contributed to the unique aroma of aromatic rice (Widjaja et al., 1996b; Yang et al., 2008). Among the compounds identified, 2-acetyl-1-pyrroline (2AP) and n-hexanal are considered to be the most important odor-active compounds (Buttery et al., 1988; Jezussek et al., 2002). 2AP had been reported to possess very low odor threshold value (a minimum detectable level) which indicates the great important contribution of this compound to aroma of the rice, though it is present in small amount (Harrison and Dake, 2005; Yang et al., 2008). Hexanal, an off-odor compound, had high odor threshold value but it was found to be the most abundant volatile compound in stored rice (Widjaja et al., 1996b; Tava and Bocchi, 1999). During storage of Thai aromatic rice cv. KDML 105, the concentration of 2AP decreased whereas hexanal increased (Laksanalamai and Ilangantileke, 1993; Wongpornchai et al., 2004). Since 2AP and hexanal play an important role in consumer acceptability, alternative postharvest technology should be sought in the way that negative effect on the appearance of these compounds can be minimized.

In this study, aroma quality and volatile components of KDML 105 freshly-harvested milled rice after being given a designed set of AA treatments were investigated whether these AA treatments could change or result in favorable or unfavorable effects on some volatile compounds that are responsible for the odor character of the aromatic rice.
MATERIALS AND METHODS

Rice samples and preparations

The rice cv. KDML 105 used in this study was grown in 2006 season at Lampang Agricultural Research and Training Center, Rajamangala University of Technology Lanna, Lampang. The rice was harvested at maturity by hand, left dry in the field for 2 to 3 days and then threshed to paddy having approximately 14% MC. The freshly-harvested paddy sample was then divided into 2 portions by a Boerner divider (Seedburo Equipment Co., Chicago, IL). One portion was de-hulled by a McGill sample sheller and milled for 30 sec in a friction-type miller operating with a 1.0 kg weight positioned at the end of a 25-cm mill lever arm. Milled head rice was separated from the broken kernel by a cylinder grader and used for the following accelerated aging treatments. The protein (N × 5.95) and lipid contents of the head rice samples were 6.54 and 0.92%, respectively, as determined by AOAC (1999) standard methods. Apparent amylose content was 17.65% (w/w) as determined by the method of Juliano et al. (1981). Moisture content of milled rice sample, determined prior to the aging treatment using oven method (103°C for 17 hr) was 13.13% (wb). The other portion of paddy sample was stored in jute sacks under ambient condition. Changes in aroma quality as measured by the amounts of 2AP and n-hexanal of its milled rice samples were monitored at 1-month interval for a storage period of 6 months.

Accelerated aging treatments

Three replicates, each of 370 g of freshly-harvested milled rice samples, were placed in aluminum containers (11 cm height \times 8.5 cm diameter) and covered with heavy-duty aluminum foil. The rice samples were then exposed to three different aging treatments, i.e., 100°C for 100 min, 110°C for 45 min and 120°C for 25 min in an automatic autoclave (SS-320, Tomy Seico Co. Ltd., Wako, Saitama, Japan). After exposure, the rice samples were left covered in the aluminum containers and cooled for about 2 hr at 21°C. Samples were then poured onto aluminum trays and their temperature and moisture contents were allowed to equilibrate with ambient air for 24 hr. Subsequently, all samples including freshly-harvested rice (control) were placed into zip-locked plastic bags and kept at -20°C until the time of each experiment.

Analysis of 2-acetyl-1-pyrroline and n-hexanal

The amounts of 2AP and n-hexanal of the AA, freshly-harvested milled rice and those stored under natural condition in rough rice form were analyzed, using the method employing headspace-gas chromatography (HS-GC) developed by Sriseadka et al., (2006). Milled rice sample was ground to pass through a 0.5 mm screen and the resulting flour, weighing exactly 1.000 g, was placed into a 20 ml headspace vial. An internal standard (1 \mu L of 0.50 mg/ml 2,6-dimethylpyridine in benzyl alcohol) was added into the vial which was then immediately sealed with a PTFE/silicone septum (Restek Corp., Bellefonte, PA) and an aluminum cap. Then, the sample vials were placed in the headspace autosampler (Agilent Technologies model G1888) of a gas chromatograph model 6890N (Agilent
Technologies, Wilmington, DE) equipped with a fused silica capillary column, HP-5 (5% phenyl 95% dimethylpolysiloxane, 30 m × 0.53 mm i.d., 1.5 μm film thickness; J&W Scientific, Folsom, CA). Sample headspace vial was equilibrated at 120°C for 9 min in the autosampler before the rice headspace was transferred to the injection port of the GC. The GC condition was set as follows: the column temperature program started at 50°C and increased at a rate of 1°C/min to 70°C, the injector and flame ionization detector (FID) temperatures were 230 and 250°C, respectively. Purified helium was used as carrier gas at a flow rate of 7 mL/min. Amounts of 2AP in the rice samples were determined by using standard calibration curves and the relative amounts of n-hexanal were derived from the ratio of the peak areas of n-hexanal and 2,6-dimethylpyridine.

Analysis of rice headspace volatile components

Volatile components in headspace of the AA and freshly harvested milled rice samples were extracted using a solid-phase microextraction (SPME) device, followed by a qualitative analysis of the volatiles by gas chromatography-mass spectrometry (GC-MS). Analysis was carried out in an Agilent Technologies (Wilmington, DE) gas chromatograph model 6890N coupled to a HP 5973 mass-selective detector (Agilent Technologies, Palo Alto, CA), and a fused silica capillary column, HP-1MS, with dimethylpolysiloxane as nonpolar stationary phase (30 m × 0.25 mm i.d. and 0.25 μm film thickness; Agilent Technologies, Wilmington, DE) was utilized. Rice flour weighed exactly 5.000 g was sealed in a 27-ml headspace vial fitted with a PTFE/silicone septum (Restek Corp., Bellefonte, PA) and an aluminum cap. The sample vial was incubated at 120°C for 45 min. A SPME fiber (Supelco, Bellefonte, PA) of 1 cm in length, coated with polydimethylsiloxane (PDMS) at 100 μm thickness mounted in the manual SPME holder (Supelco) was then inserted through the septum of the vial. The fiber was allowed to absorb volatile compounds in the headspace for 15 min while temperature of the sample was still held at 120°C. Then, the SPME fiber was withdrawn from the sample vial and volatile components were desorbed at 250°C in the GC-MS injection port prior to the component separation and analysis by GC-MS.

The GC-MS condition was set as follows: injection port was in splitless mode; initial column temperature, 45°C; ramped at a rate of 2°C /min to 180°C; mass spectrometer was operated in the electron impact (EI) mode with an electron energy of 70 eV; ion source temperature, 230°C; quadrupole temperature, 150°C; mass range, m/z 29-550; scan rate, 0.68 s/scan; EM voltage, 1423 V. The GC-MS transfer line was set to 280°C and purified helium gas at a flow rate of 1 mL/min was used as the carrier gas. The volatile compounds were tentatively identified by comparing their mass spectra with those compiled in the Wiley7n and NIST 98 libraries of the MS database.

Statistical analysis

Data regarding the quantities of 2AP and n-hexanal of KDML 105 rice samples were analyzed using analysis of variance (ANOVA) to determine the effect of AA treatments. Differences among samples were determined by least
significant difference test (LSD) at $P<0.05$.

RESULTS AND DISCUSSION

Aroma quality on the basis of 2-acetyl-1-pyrroline and $n$-hexanal contents

The amounts of 2AP in the rice samples decreased after AA treatment (Figure 1). The concentrations obtained from rice aged with 100°C for 100 min, 110°C for 45 min and 120°C for 25 min were 3.33, 3.78 and 3.94 ppm, respectively. The 100°C-100 min treatment had the highest percent of reduction (33.9%) whereas 120°C-25 min had only 21.8% when calculated on the basis of 2AP content (5.04 ppm) of freshly-harvested milled rice. These results revealed that reduction of 2AP was greater in rice given longer duration treatment, although the heating temperature applied to the rice was lower (100°C for 100 min). In comparison with those naturally-aged rice,

![Figure 1. Effect of accelerated aging treatments (temperature and duration) on concentration of the aroma compound, 2-acetyl-1-pyrroline, in KDML105 milled rice samples. Control = freshly-harvested KDML 105 milled rice. Vertical bars (±SD) with the same letters are not significantly different at $P<0.05$, LSD.](image)

the contents of 2AP in all AA samples were higher than that observed in 3-month naturally-stored sample (2.95 ppm) (Figure 2). Thus, 2AP of the naturally-stored samples decreased rapidly and were lower than those of the AA rice after storage for 3 months.
Figure 2. 2-Acetyl-1-pyrroline concentrations in KDML105 milled rice stored as paddy in ambient condition for a period of 6 months. Vertical bars (±SD) with the same letters are not significantly different at $P<0.05$, LSD.

Consequently, the age-accelerated treatment using high temperature and short duration ($120^\circ$C for 25 min) would be recommended for the production of KDML 105 AA rice. The high 2AP content in the rice aged by this heating condition might be attributed to a shorter duration of heating time, being not sufficient for the release of 2AP from inner part of the rice kernel to its surrounding atmosphere. Thus, a large portion of 2AP still remained in the rice kernel. Analysis of 2AP in the rice kernel by the previous studies revealed that the compound was equally distributed across kernel of aromatic rice (Bergman et al., 2000) and it was reported to be present in the starch granule of milled rice kernel in both free and starch-bound forms, with the latter required higher temperature and more time for extraction (Yoshihashi et al., 2005). These research findings could support the aforementioned postulation. 2AP is formed naturally in rice during growth in paddy field (Yoshihashi et al., 2002) and its concentration decreases with time of storage (Wongpornchai et al., 2004; Yoshihashi et al., 2005). Our results suggest the advantage of AA technique to bring the freshly-harvested rice to an advanced stage of aging, yielding rice of similar cooking quality to that of stored rice while still maintaining its high aroma quality.

During processing, the relative amounts of n-hexanal in AA samples were reduced significantly (Figure 3). Area ratios of n-hexanal/DMP of the AA samples were in the range of 0.37 to 0.47 which were lower than that of the freshly-harvested milled rice (0.60). Analysis was also made to observe the amounts of n-hexanal generated on those rice stored in paddy form under natural condition (Figure 4). The results revealed that the relative contents of n-hexanal in the naturally-stored rice samples were higher than those of the rice given AA treatments. The increase in n-hexanal of naturally-stored rice samples was attributed to the degradation of lipid composition of the rice. Lipids in rice were reported to be hydrolyzed and...
Figure 3. Effect of accelerated aging treatments (temperature and duration) on the relative content of n-hexanal in KDML105 milled rice samples. Control = freshly-harvested KDML 105 milled rice. Vertical bars (±SD) with the same letters are not significantly different at $P<0.05$, LSD.

oxidized to free fatty acids or peroxides (Zhou et al., 2002), which subsequently resulted in the increases in some off-odor compounds, including n-hexanal of the stored rice. This carbonyl compound, $n$-hexanal, was reported to be a degradation product of linoleic acid (Monsoor and Proctor, 2004). Age-accelerated technique, using high temperature in this study, might affect the activity of lipid hydrolysis enzyme and, at the same time, enhance the release of this compound, resulting in lower content of $n$-hexanal in the AA samples which indicated that aroma quality of the AA rice was improved.

Figure 4. Change in area ratios of $n$-hexanal/DMP of KDML105 milled rice stored as paddy in ambient condition for a period of 6 months. Vertical bars (±SD) with the same letters are not significantly different at $P<0.05$, LSD.
Aroma quality on the basis of volatile components as determined by GC-MS

Gas chromatographic profiles of volatile components of the freshly-harvested milled rice and its corresponding AA samples are illustrated in Figures 5, 6 and 7. These volatile components were extracted from headspace of milled rice samples using SPME technique. Following the analysis of total rice volatiles by GC-MS, the overall aroma quality of the AA rice samples was assessed on the basis of the similarity of their volatile component profiles compared with that of the freshly-harvested milled rice. All of the volatile compounds typically present in the headspace of KDML105 rice showed themselves in the chromatograms of

<table>
<thead>
<tr>
<th>Peaks</th>
<th>Component Identification</th>
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<tbody>
<tr>
<td>1</td>
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<td>2</td>
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<td>16</td>
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</table>

*Peaks labeled (*) are those interferences resulted from degradation of the SPME adsorbent.

**Figure 5.** GC-MS chromatograms of KDML 105 freshly-harvested milled rice (FR) and after being given an accelerated aging (AA) at 100°C for 100 min. Numbers above the peaks indicate the component identification. Peaks labeled (*) are those interferences resulted from degradation of the SPME adsorbent.
Figure 6. GC-MS chromatograms of KDMIL 105 freshly-harvested milled rice (FR) and after being given an accelerated aging (AA) at 110°C for 45 min. Numbers above the peaks indicate the component identification. Peaks labeled (*) are those interferences resulted from degradation of the SPME adsorbent.

the AA rice samples. There were no additional compounds generated or formed as a consequence of the AA treatments. Each chromatogram showed 13 identified components (Table 1), which were classified as aldehydes (\(n\)-hexanal, \(n\)-heptanal, benzaldehyde, \(n\)-nonanal, and \(n\)-decanal), alcohols (1-octen-3-ol and 1-octanol), hydrocarbons (\(n\)-dodecane, \(n\)-tridecane, (E)-2-tetradecene and \(n\)-tetradecane) and heterocyclic compounds (2-acetyl-1-pyrroline and 2-pentylfuran). Among the compounds identified, \(n\)-nonanal was found to be the most abundant compound in headspace of both AA and freshly harvested rice samples, followed by benzaldehyde and \(n\)-hexanal.
Figure 7. GC-MS chromatograms of KDML 105 freshly-harvested milled rice (FR) and after being given an accelerated aging (AA) at 120°C for 25 min. Numbers above the peaks indicate the component identification. Peaks labeled (*) are those interferences resulted from degradation of the SPME adsorbent.

During processing, high temperature of AA treatments could likely promote oxidation of the rice constituents and concurrently enhance some portion of these highly-volatile compounds to be released from the rice kernel. These occurrences led to the reduction in quantities of volatiles in headspace of the milled rice samples as observed by the decreases in peak areas ratio of some rice volatiles in the chromatograms of AA samples (Table 1). Suggestion had been made that the unique aroma characteristic of fragrant rice is dependent on the levels and the relative proportions of many individual components that make up its fragrance characteristic (Widjaja et al., 1996a). Results of this study revealed that the decreases in the levels of volatile components in AA rice were indirect proportion with the contents of their respective compounds in freshly-harvested rice,
and among AA samples. However, aging at 120°C for 25 min showed minimum reduction of peak areas of the rice volatiles. Although relative contents of the rice volatiles were reduced by the AA process, reasonable amounts still remained which indicated that the overall aroma quality of the AA rice samples was not affected.

Table 1. Identification of volatile components in freshly-harvested and accelerated-aged KDML 105 milled rice.

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>RTa</th>
<th>Compound</th>
<th>m/z$^b$</th>
<th>Match quality (%)</th>
<th>MW$^c$</th>
<th>Peak area ratios$^d$</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FR, H100, H110, H120</td>
</tr>
<tr>
<td>1</td>
<td>3.22</td>
<td>n-hexanal</td>
<td>100(2), 85(4), 82(26), 72(28), 67(16), 57(68), 56(96), 44(100)</td>
<td>90</td>
<td>100</td>
<td>11.41±0.85, 5.43±0.07, 7.13±0.09, 8.25±0.10</td>
</tr>
<tr>
<td>2</td>
<td>5.23</td>
<td>2,6-dimethylpyridine$^e$</td>
<td>107(100), 106(45), 92(15), 79(10), 66(19), 44(30), 40(17)</td>
<td>89</td>
<td>107</td>
<td>- - -</td>
</tr>
<tr>
<td>3</td>
<td>5.52</td>
<td>n-heptanal</td>
<td>114(3), 96(17), 86(16), 81(33), 70(100), 68(17), 57(38), 55(59)</td>
<td>93</td>
<td>114</td>
<td>2.31±0.18, 1.14±0.07, 1.23±0.08, 1.54±0.04</td>
</tr>
<tr>
<td>4</td>
<td>6.21</td>
<td>2-acetyl-1-pyrroline</td>
<td>111(24), 83(41), 69(22), 68(21), 55(4), 52(4), 43(100), 41(53)</td>
<td>86</td>
<td>111</td>
<td>2.01±0.27, 0.90±0.04, 1.09±0.18, 1.55±0.10</td>
</tr>
<tr>
<td>5</td>
<td>7.55</td>
<td>benzoaldehyde</td>
<td>106(100), 105(98), 78(17), 77(88), 51(34), 50(20)</td>
<td>97</td>
<td>106</td>
<td>19.35±1.21, 15.61±0.96, 14.20±0.43, 17.16±0.69</td>
</tr>
<tr>
<td>6</td>
<td>8.42</td>
<td>1-octen-3-ol</td>
<td>128(2), 99(7), 85(12), 82(8), 72(16), 67(100), 57(100), 55(16)</td>
<td>90</td>
<td>128</td>
<td>1.67±0.11, 0.61±0.03, 0.67±0.01, 0.91±0.08</td>
</tr>
<tr>
<td>7</td>
<td>8.89</td>
<td>2-pentyl furan</td>
<td>138(10), 109(7), 95(7), 82(23), 81(100), 53(14), 44(14), 41(14)</td>
<td>92</td>
<td>138</td>
<td>2.72±0.12, 1.64±0.14, 2.02±0.18, 1.90±0.06</td>
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<tr>
<td>8</td>
<td>10.93</td>
<td>benzy alcohol</td>
<td>108(99), 107(70), 91(17), 79(100), 77(64), 65(7), 51(21)</td>
<td>97</td>
<td>108</td>
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<tr>
<td>9</td>
<td>12.93</td>
<td>1-octanol</td>
<td>130(1), 112(4), 84(68), 83(49), 70(68), 69(83), 57(46), 56(100), 55(85), 43(66), 42(46), 41(92)</td>
<td>89</td>
<td>130</td>
<td>5.11±0.24, 1.24±0.14, 2.09±0.08, 9.29±0.14</td>
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<td>10</td>
<td>14.71</td>
<td>n-nonanal</td>
<td>142(2), 124(4), 114(9), 98(47), 95(31), 82(36), 70(44), 57(100), 44(45), 41(82)</td>
<td>91</td>
<td>142</td>
<td>7.93±0.30, 16.66±1.09, 22.61±0.55, 35.66±0.88</td>
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<td>11</td>
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<td>n-dodecane</td>
<td>170(12), 141(2), 113(3), 85(47), 71(100), 43(67)</td>
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<td>2.19±0.08, 1.00±0.07, 1.29±0.06, 1.95±0.08</td>
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<tr>
<td>12</td>
<td>20.82</td>
<td>n-decanal</td>
<td>156(2), 122(30), 109(11), 95(42), 82(69), 71(68), 67(57), 57(100)</td>
<td>90</td>
<td>156</td>
<td>2.98±0.20, 0.72±0.01, 1.42±0.02, 2.00±0.07</td>
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<tr>
<td>13</td>
<td>25.49</td>
<td>unknown</td>
<td>114(1), 85(80), 84(19), 71(100), 69(14), 57(99), 43(68)</td>
<td>- -</td>
<td>-</td>
<td>2.02±0.16, 0.80±0.07, 1.18±0.04, 1.44±0.07</td>
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<tr>
<td>14</td>
<td>26.79</td>
<td>n-tridecane</td>
<td>186(10), 127(8), 112(9), 99(10), 85(45), 71(62), 57(100), 43(86)</td>
<td>94</td>
<td>184</td>
<td>4.51±0.12, 2.83±0.29, 2.84±0.05, 4.06±0.10</td>
</tr>
<tr>
<td>15</td>
<td>32.47</td>
<td>(E)-2-tetradecene</td>
<td>198(16), 111(48), 97(81), 83(93), 69(88), 55(100), 41(97)</td>
<td>97</td>
<td>196</td>
<td>5.11±0.17, 2.17±0.16, 2.80±0.04, 4.53±0.22</td>
</tr>
<tr>
<td>16</td>
<td>32.97</td>
<td>n-tetradecane</td>
<td>198(7), 127(9), 99(10), 85(50), 71(73), 57(100), 43(58)</td>
<td>93</td>
<td>198</td>
<td>6.23±0.63, 3.27±0.23, 3.73±0.04, 5.22±0.16</td>
</tr>
</tbody>
</table>

$^a$RT, Retention time (min); $^b$m/z, mass/charge ratio; $^c$MW, Molecular weight; $^d$Peak area ratio of each compound and 2,6-dimethylpyridine (internal standard); $^e$FR, Freshly-harvested rice; H100, 100°C-100 min; H110, 110°C-45 min; H120, 120°C-25 min; Internal standard; $^f$Solvent of internal standard. Data represent the average±standard deviation of three determinations.
CONCLUSION

Based on the results of volatile analysis by HS-GC and SPME-GC-MS of the milled rice samples, it can be concluded that the profiles of volatile constituents making up the odor character of rice cv. KDML 105 given accelerated aging were not different from that of the ordinary freshly-harvested rice. Though there were decreases in relative contents of the volatile components, all the volatile compounds found in freshly-harvested rice were present in the AA rice samples. Moreover, the AA rice had better aroma quality than that of 3-month naturally-aged rice in terms of higher amount of the key aroma compound, 2AP, and lower content of an off-odor, n-hexanal, present in their kernels.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Postharvest Technology Research Institute, Postharvest Technology Innovation Center, Chiang Mai University, for financial and laboratory facility support, and the Center of Excellence for Innovation in Chemistry, Commission on Higher Education, Ministry of Education, for its support in lending the HS-GC and GC-MS instruments. Our special thanks are given to Mr. Tinakorn Sriseadka for his assistance.

REFERENCES


Effectiveness of Sodium Hypochlorite, Peroxyacetic Acid and Peroxycitric Acid in Reducing Microorganisms on the Surface of Fresh Whole Litchi Fruit and Its Arils

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ABSTRACT

The effectiveness of three sanitizers, sodium hypochlorite (NaOCl), peroxycetic acid (PAA) and peroxycitric acid (PCA) in decreasing the total number of bacteria and yeast-molds on the peel of whole litchi fruit and its arils of three cultivars, cv. Honghuay, Gimjeng and Jugkapat were studied. First, the optimal concentration and treatment time of PAA and PCA were determined for whole litchi fruit (concentrations: 75, 100, 150 or 200 mg/L; treatment times: 1, 3 or 5 min) and for the arils (concentrations: 50 or 75 mg/L; treatment times: 1 or 3 min). Treatments were compared with undipped and dipped controls in tap water. The best treatments of PAA and PCA for sanitizing three cultivars of whole litchi fruit were 100 mg/L for 5 min and 200 mg/L for 3 min, respectively. For the arils, the best treatments of PAA and PCA for three cultivars were 50 mg/L for 1 min and 50 mg/L for 3 min, respectively. The effectiveness of PAA and PCA were then compared with NaOCl at a commercial recommendation levels (concentrations: 200 and 50 mg/L; treatment times: 3 min). The results showed that PAA was the most efficient in reducing microorganisms on whole litchi fruit and arils when compared with NaOCl and PCA. Therefore, PAA could be a potential alternative to NaOCl or chlorine as sanitizer for whole litchi fruit and its arils.

Key words: Sodium hypochlorite, Peroxyacetic acid, Peroxycitric acid, Litchi

INTRODUCTION

Litchi (Litchi chinensis Sonn.) is a subtropical Asian fruit with a natural red color, sweet acidic taste and aroma. The fruit has a high commercial value in the international market. The major factors reducing the storage life and marketability of fruit are microbial decay and browning of outer covering pericarp within 2-3 days after harvest at 20°C (Holcroft and Mitcham, 1996; Jieng, 2003). Thus, litchi
fruits are rejected by the market even though the edible arils (white, translucent, firm and juicy tissue covered by the pericarp) still remain in excellent condition. Arils or flesh of such litchi fruit can be preserved by minimal processing which provides fresh-like fruit with simplicity in use and convenience (Shah and Nart, 2008).

In minimal processing, washing of outer pericarp can reduce the overall potential for microbial food safety hazards because most microbial contamination is on the surface of the fruit. Final washing of arils after peeling helps remove some of the cellular fluids that could serve as a nutrient for microbial growth (USFDA, 2006). Chlorine is normally used for the disinfection of whole and fresh-cut fruit. Dipping in chlorine water containing 50-200 mg/L of free chlorine (recommended concentrations) is commonly used (Soliva-Fortuny and Martin-Bellos, 2003). Moreover, it is known that the reaction of chlorine with natural organic matter results in the formation of carcinogenic halogenated by-products (DBP), like trihalomethanes (THMs) and haloacetic acids (HAAs) (Artes et al., 2009). Use of chlorine is also associated with the production of high amounts of waste water with very high levels of biological oxygen demand (BOD) (Olmez and Kretzchmar, 2008). Due to the above described problems, alternative sanitizing agents to replace chlorine have gained much interest in recent years.

Peroxides such as peracetic acid (PAA) for sanitizing fruit and vegetables as an alternative to chlorine has been used on apples (Wisniewsky, 2000), lettuce (Beuchat et al., 2004; Kim et al., 2006), stone fruit (Mari et al., 2004) and whole mango fruit and flesh (Narciso and Plotto, 2005). Moreover, percitric acid (PCA) is one of the organic peroxides (Ferdousi et al., 2007, 2008). However, effectiveness of PCA in reducing microbial populations on fresh fruit and vegetable has not been reported. The objective of this study was to 1) determine the most effective concentration and treatment time of PAA and PCA in controlling microorganisms on whole litchi fruit and the arils of different cultivars and 2) using the most effective concentration and treatment time of the two sanitizers and compare its effectiveness with that of NaOCl of whole fruit and arils of different cultivars.

MATERIALS AND METHODS

Fruit

Litchi (Litchi chinensis Sonn.) fruit cv. Honghuay, Gimjeng and Jugkapat, at the fully-red color and commercially-harvesting stage were purchased from 3 different retailers in Chiang Mai Province, Thailand, during June-August, 2008. Fruit were then kept at 4±1°C overnight and selected for uniformity of size, shape, color and lack of physical damage and injury caused by insects, prior to use in the following two experiments.
Experiment 1. Determining the most effective concentration and treatment time of PAA and PCA for sanitizing whole litchi fruit and arils.

For whole litchi fruit, the experiment was designed as 4x3 factorial in Completely Randomized Design (CRD) with 3 replicates, 4 levels of concentration and 3 levels of treatment time. Five fruit per replication were dipped in 75, 100, 150 or 200 mg/L of PAA or PCA for 1, 3 or 5 min. After draining, fruit were analyzed for total bacteria (BAM, 2001) and yeast-molds (AOAC, 2000). Treatments were compared with undipped and dipped controls in tap water. Litchi fruit from three different locations were used.

For arils, the experiment was designed as 2x2 factorial in CRD with 3 replicates, 2 levels of concentration and 2 levels of treatment time. Five fruit per replication were washed with the best treatment of sanitizer for whole fruit. The seed was removed with sanitized sharp-point knife prior peeling. Then, five arils per replicate were dipped in 50 or 75 mg/L of PAA or PCA for 1 or 3 min. After draining, arils were evaluated for total bacteria (BAM, 2001) and yeast-molds (AOAC, 2000). Treatments were compared with undipped and dipped controls in tap water. Litchi fruit from three different locations were used.

Experiment 2. Comparison of PAA and PCA to NaOCl for sanitizing whole litchi fruit and arils.

PAA and PCA were compared against NaOCl in reducing total bacteria and yeast-mold populations on whole litchi fruit and arils of different cultivars using CRD with 3 replicates.

For whole litchi fruit, five fruit per replication were dipped in NaOCl at a commercially recommended level (concentration 200 mg/L for 3 min) or the best treatment of PAA and PCA obtained from Experiment 1. After draining, fruit were evaluated for total bacteria (BAM, 2001), and yeast-molds (AOAC, 2000).

For arils, five fruit per replication were washed with the best treatment of sanitizer for whole fruit. The seed was removed with sanitized sharp-point knife prior peeling. Then, five arils per replicate were dipped in NaOCl at a commercial recommendation level (concentration 50 mg/L for 3 min) or the best treatment of PAA and PCA obtained from Experiment 1. After draining, fruit were analyzed for total bacteria (BAM, 2001) and yeast-molds (AOAC, 2000).

Preparation of sanitizers

Chlorinated water (concentration 200 and 50 mg/L) was prepared with Clorox® USA (5.7% chlorine), adjusted to pH 6.5 with 50% citric acid. Five concentration levels 50, 75, 100, 150 and 200 mg/L of PAA at pH 2.55-3.54 were prepared from PAA solution (PAA 5%; Thaiperoxide Co., Ltd, Thailand). Five concentration levels of PCA 50, 75, 100, 150 and 200 mg/L at pH 2.30-4.42 were prepared from PCA solution (PCA 5%; Thaiperoxide Co., Ltd, Thailand).
**Determination of microbial population**

For whole litchi fruit, for each treatment, five fruit were transferred to a sterilized bag containing 50 ml of 0.1% phosphate buffer pH 7.2. Five fruit and phosphate buffer in bags were firmly hand-rubbed for 2 min. Samples were serially-diluted by a factor of ten in phosphate buffer. The undiluted mixture and serially-diluted mixture (0.1 ml in duplicate) were spread on plate count agar and potato dextrose agar for total bacteria and yeast-molds count, respectively. Then, plate count agar and potato dextrose agar were incubated at 35°C for 48 hr and 25°C for 48 hr, respectively. Values are reported as log CFU/fruit.

Arils from five fruit per replicate were cut with sterilized stainless steel scissors and a 10 g sample was weighed for analysis. The samples were transferred to a sterilized bag containing 90 ml of 0.1% phosphate buffer pH 7.2 and samples were macerated by stomacher (IVL Masticator 400, Spain) for 30 sec. The homogenized samples were serially-diluted by a factor of ten in phosphate buffer. The undiluted mixture and serially diluted mixture (0.1 ml in duplicate) were spread on plate count agar and potato dextrose agar for total bacteria and yeast-molds count, respectively. Then, plate count agar and potato dextrose agar were incubated at 35°C for 48 hr and 25°C for 48 hr, respectively. Values are reported as log CFU/g.

**Statistical analysis**

All experiments were replicated three times. Triplicate samples were analyzed and diluted samples were plated in duplicate (total n=18). Data were analyzed using SPSS program V.13 for analysis of variance. Duncan’s multiple range test was used for comparison of means to determine differences in microbial counts for treatments.

**RESULTS AND DISCUSSION**

**Effectiveness of PAA on three cultivars of whole litchi fruit**

The number of total bacteria and yeast-molds initially on the surface of whole litchi fruit were 6.35-6.77 log CFU/fruit and 6.13-6.47 log CFU/fruit, respectively. Tap water treatment did not cause a significant change in total bacteria and yeast-molds count when compared with undipped control, the microbial reduction was less than 0.5 log CFU/fruit (data not shown). For treatment time of 1 or 3 min of whole fruit, 200 mg/L PAA achieved the highest reductions on total bacteria by 1.84 and 1.80 log CFU/fruit in Honghuay, 1.95 and 2.40 log CFU/fruit in Gimjeng and 1.56 and 2.23 log CFU/fruit in Jugkapat, respectively, and also yeast-molds by 1.84 and 1.83 log CFU/fruit in Honghuay, 2.32 and 2.63 log CFU/fruit in Gimjeng and 1.57 and 2.29 log CFU/fruit in Jugkapat, respectively (Table 1). The 5 min treatment time was more effective than the 1 or 3 min in reducing the total bacteria and yeast-mold populations, and no differences were noted among the concentration treatments with all three litchi cultivars. Therefore, the lowest concentration at 5 min, i.e., 100 mg/L PAA for 5 min was used for sanitation treatment in subsequent experiments.
Table 1. Log reductions of total bacteria and yeast-molds from whole litchi fruit treated with PAA at different concentrations and treatment times.

<table>
<thead>
<tr>
<th>Treatment times (min)</th>
<th>Conc. (mg/L)</th>
<th>Log reductions (log CFU/fruit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Honghuay</td>
<td>Gimjeng</td>
</tr>
<tr>
<td>1</td>
<td>75</td>
<td>0.30 h</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>1.34 f</td>
</tr>
<tr>
<td>1</td>
<td>150</td>
<td>1.43 ef</td>
</tr>
<tr>
<td>1</td>
<td>200</td>
<td>1.84 b</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>0.51 g</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>1.55 de</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>1.64 cd</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>1.59 de</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>2.22 a</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>2.25 a</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>2.21 a</td>
</tr>
</tbody>
</table>

TBC = Total bacteria count, Y&M = Yeast and molds, Values are means ± SD of n = 18. Populations of TBC on undipped control were 6.51, 6.35 and 6.77 log CFU/fruit on Honghuay, Gimjeng and Jugkapat, respectively. Populations of Y&M on undipped control were 6.20, 6.13 and 6.47 log CFU/fruit on Honghuay, Gimjeng and Jugkapat, respectively. Values in each column with distinct letters represent the significantly different results (p < 0.05).

Effectiveness of PAA as a sanitizer is based on the release of active oxygen, which oxidize sensitive sulfhydryl and sulfur bonds in proteins, enzymes and other metabolites of the bacteria and yeast-molds (Kitis, 2004).

Effectiveness of PCA on three cultivars of whole litchi fruit

The number of total bacteria and yeast-molds initially on the surface of whole litchi fruit were 6.53-6.82 log CFU/fruit and 6.11-6.33 log CFU/fruit, respectively. Tap water treatment did not cause a significant change in total bacteria and yeast-molds count when compared with undipped control, the reduction was less than 0.5 log CFU/fruit (data not shown). Total bacteria and yeast-molds were reduced by less than 0.80 log CFU/fruit when whole litichi fruit of all three cultivars were dipped in 75, 100 or 150 mg/L PCA at all treatment times (Table 2). The microorganisms were reduced by more than 0.80 log CFU/fruit; approximately 1.03 log CFU/fruit, when treated with 200 mg/L PCA for 3 or 5 min. Therefore, the 3 min treatment with 200 mg/L PCA was used for sanitation treatment in subsequent experiments.

Effectiveness of three sanitizers on whole litchi fruit

The effectiveness of the three sanitizers used in this study were compared (Table 3). The highest reduction resulted from treatment of whole litchi fruit with 100 mg/L PAA for 5 min, achieving reductions of total bacteria and yeast-molds by 2.44 and 2.67 log CFU/fruit in Jugkapat, 2.32 and 2.57 log CFU/fruit in Gimjeng...
and 2.25 and 2.45 log CFU/fruit in Honghuay, respectively, followed by treatment with 200 mg/L NaOCl for 3 min where reduction was less than 1.5 log CFU/fruit. Treatment with 200 mg/L PCA for 3 min was less effective, reductions did not exceed 1.3 log CFU/fruit (Table 3). These results agree with those of Narciso and Plotto (2005) who showed that 100 mg/L PAA was more effective than 200 mg/L NaOCl on the reduction of microbial populations in whole mango fruit.

**Effectiveness of PAA on three cultivars of litchi arils**

The numbers of total bacteria and yeast-molds initially on the surface of arils were 3.63-3.93 log CFU/g and 3.39-3.59 log CFU/g, respectively. Treatment with PAA at two levels of concentration or two levels of treatment time were not significant ($p \leq 0.05$) in reducing the microorganism populations. Total bacteria and yeast-molds were reduced by 1.30 and 1.50 log CFU/g in Honghuay, 1.70 and 2.20 log CFU/g in Gimjeng and 1.70 and 1.70 log CFU/g in Jugkapat, respectively (Table 4). Therefore, 50 mg/L PAA for 1 min was the optimal treatment for the three cultivars of litchi arils.

In contrast, Kim (2006) reported that the 3 min treatment was more effective than the 1 min time in reducing the *Enterobacter sakazakii* population when shredded lettuce were treated with 40 or 80 mg/L PAA. The reason for difference is not known. No differences were found between the two concentration treatments.

**Table 2.** Log reductions of total bacteria and yeast-molds from whole litchi fruit treated with PCA at different concentrations and treatment times.

<table>
<thead>
<tr>
<th>Treatment times (min)</th>
<th>Conc. (mg/L)</th>
<th>Log reductions (log CFU/fruit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Honghuay</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TBC</td>
</tr>
<tr>
<td>1</td>
<td>75</td>
<td>0.07 e</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>0.49 d</td>
</tr>
<tr>
<td>1</td>
<td>150</td>
<td>0.59 cd</td>
</tr>
<tr>
<td>1</td>
<td>200</td>
<td>0.67 bcd</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>0.19 e</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>0.56 cd</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>0.81 abc</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>0.52 d</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>0.80 abc</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>0.86 ab</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>1.03 a</td>
</tr>
</tbody>
</table>

TBC = Total bacteria count, Y&M = Yeast and molds, Values are means ± SD of n = 18. Populations of TBC on undipped control were 6.53, 6.37 and 6.82 log CFU/fruit on Honghuay, Gimjeng and Jugkapat, respectively. Populations of Y&M on undipped control were 6.15, 6.11 and 6.33 log CFU/fruit on Honghuay, Gimjeng and Jugkapat, respectively. Values in each column with distinct letters represent the significantly different results ($p \leq 0.05$).
Table 3. Populations of total bacteria and yeast-molds recovered from whole litchi fruit when treated with three types of sanitizers.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Experiment units</th>
<th>Microbial populations (log CFU/fruit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Honghuay</td>
<td>Gimjeng</td>
</tr>
<tr>
<td>Total bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg/L PAA for 5 min</td>
<td>3.55 ± 0.12 c</td>
<td>4.07 ± 0.09 b</td>
</tr>
<tr>
<td>200 mg/L PCA for 3 min</td>
<td>4.87 ± 0.03 a</td>
<td>5.40 ± 0.15 a</td>
</tr>
<tr>
<td>200 mg/L NaOCl for 3 min</td>
<td>4.43 ± 0.24 b</td>
<td>5.49 ± 0.04 a</td>
</tr>
<tr>
<td>Yeast &amp; molds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg/L PAA for 5 min</td>
<td>3.45 ± 0.16 c</td>
<td>3.79 ± 0.10 b</td>
</tr>
<tr>
<td>200 mg/L PCA for 3 min</td>
<td>5.09 ± 0.05 a</td>
<td>5.32 ± 0.07 a</td>
</tr>
<tr>
<td>200 mg/L NaOCl for 3 min</td>
<td>4.76 ± 0.12 b</td>
<td>5.29 ± 0.05 a</td>
</tr>
</tbody>
</table>

Values are means ± SD of n = 18.

Populations of total bacteria on undipped control were 5.76, 6.39 and 6.47 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively.

Populations of yeast and molds on undipped control were 5.90, 6.30 and 6.42 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively.

Values in each column with distinct letters represent the significantly different results (p ≤ 0.05).

Table 4. Log reductions of total bacteria and yeast-molds from arils treated with PAA at different concentrations and treatment times.

<table>
<thead>
<tr>
<th>Treatment times (min)</th>
<th>Conc., (mg/L)</th>
<th>Log reductions (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBC</td>
<td>Y&amp;M</td>
</tr>
<tr>
<td>Honghuay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>1.36 ns</td>
</tr>
<tr>
<td>1</td>
<td>75</td>
<td>1.33 ns</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>1.39 ns</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>1.39 ns</td>
</tr>
</tbody>
</table>

TBC = Total bacteria count, Y&M = Yeast and molds, Values are means ± SD of n = 18.

Populations of TBC on undipped control were 3.63, 3.87 and 3.93 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively.

Populations of Y&M on undipped control were 3.39, 3.80 and 3.59 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively.

Values in each column with distinct letters represent the significantly different results (p ≤ 0.05).

Effectiveness of PCA on three cultivars of litchi arils

The number of total bacteria and yeast-molds initially on the surface of arils were 3.60-3.96 log CFU/g and 3.30-3.73 log CFU/g, respectively. Tap water treatment did not cause a significant change in total bacteria and yeast-mold count when compared with undipped control (data not shown). Treatment with PCA at two levels of concentration were not significant (p< 0.05) on the microorganism populations (Table 5). However, dipping the arils in PCA at all concentrations for 3 min did reduce microorganism populations than the 1 min treatment. Total bacteria and yeast-molds in Honghuay, Gimjeng and Jugkapat cultivars were reduced by 0.97, 1.04, 1.27 log CFU/g and 1.06, 1.13, 1.24 log CFU/g, respectively (Table 5). Therefore, the best treatment of PCA for sanitizing three cultivars of litchi arils was 50 mg/L for 3 min.
However, the effectiveness of PCA as a sanitizer for fruit and vegetable has not been reported. The results from this work showed that effectiveness of PCA was equivalent in the reduction of mesophilic bacteria on fresh-cut lettuce with dipping in 1% citric acid, which was reduced by 1.50 log CFU/g (Akbas and Olmez, 2007).

<table>
<thead>
<tr>
<th>Treatment times (min)</th>
<th>Conc. (mg/L)</th>
<th>Log reductions (log CFU/g)</th>
<th>Honghuay</th>
<th>Gimjeng</th>
<th>Jugkapat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TBC Y&amp;M TBC Y&amp;M TBC Y&amp;M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>0.74 b 0.80 b 0.89 b 0.97 b 1.06 b 1.07 b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>75</td>
<td>0.77 b 0.85 b 0.90 b 1.02 b 1.03 b 1.09 b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>0.95 a 1.01 a 1.04 a 1.11 a 1.27 a 1.24 a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>0.98 a 1.10 a 1.04 a 1.15 a 1.26 a 1.24 a</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TBC = Total bacteria count, Y&M = Yeast and molds, Values are means ± SD of n = 18. Populations of TBC on undipped control were 3.60, 3.85 and 3.96 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively. Populations of Y&M on undipped control were 3.30, 3.66 and 3.73 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively. Values in each column with distinct letters represent the significantly different results (p ≤ 0.05).

Effectiveness of three sanitizers on arils
The effectiveness of the three sanitizers used in this study were statistically different (p ≤ 0.05). Treatment with 50 mg/L PAA for 1 min was the most effective among the treatments, which reduced the number of total bacteria by 1.58, 2.06, 1.91 log CFU/g and 1.73, 2.25, 1.96 log CFU/g on yeast-molds in Honghuay, Gimjeng and Jugkapat, respectively (Table 6). PCA treatment with 50 mg/L for 3 min was the least effective in reducing microbial populations, followed by 50 mg/L NaOCl for 3 min and 50 mg/L PAA for 1 min. These results agree with those of Ruiz-Cruz et al., (2007) who showed that 40 mg/L PAA was more effective than 200 mg/L NaOCl on the reduction of *Salmonella* spp. in fresh-cut carrot under processed water condition (COD 35,000 mg/L).

CONCLUSION
Results from this work showed that 100 mg/L PAA for 5 min and 50 mg/L PAA for 1 min are an alternative to NaOCl treatment for sanitizing three cultivars of whole litchi fruit and its arils, respectively. PAA has the advantage of being more stable and can preserve its efficacy even in the presence of organic matter. It caused higher reduction of total bacteria and yeast-molds.
ACKNOWLEDGEMENTS

The financial support for this research was provided by Enhancing the Values of Economic Fruit Products for upland sustainable agricultural development, Faculty of Agro-Industry, Chiang Mai University. Thaiperoxide Co., Ltd., supplied PAA and PCA. We especially thank Dr. A.E. Watada for advice on the manuscript.

REFERENCES


Table 6. Populations of total bacteria and yeast-molds recovered from arils when treated with three types of sanitizers.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Experiment units</th>
<th>Microbial populations (log CFU/fruit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Honghuay</td>
<td>Gimjeng</td>
</tr>
<tr>
<td>Total bacteria</td>
<td>50 mg/L PAA for 1 min</td>
<td>2.23 ± 0.08 c</td>
</tr>
<tr>
<td></td>
<td>50 mg/L PCA for 3 min</td>
<td>2.87 ± 0.19 a</td>
</tr>
<tr>
<td></td>
<td>50 mg/L NaOCl for 3 min</td>
<td>2.55 ± 0.08 b</td>
</tr>
<tr>
<td>Yeast &amp; molds</td>
<td>50 mg/L PAA for 1 min</td>
<td>2.02 ± 0.10 c</td>
</tr>
<tr>
<td></td>
<td>50 mg/L PCA for 3 min</td>
<td>2.73 ± 0.13 a</td>
</tr>
<tr>
<td></td>
<td>50 mg/L NaOCl for 3 min</td>
<td>2.33 ± 0.06 b</td>
</tr>
</tbody>
</table>

Values are means ± SD of n = 18.

Populations of total bacteria on undipped control were 3.81, 4.13 and 4.40 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively.

Populations of yeast and molds on undipped control were 3.75, 3.94 and 4.06 log CFU/g on Honghuay, Gimjeng and Jugkapat, respectively.

Values in each column with distinct letters represent the significantly different results (p < 0.05).


Official Methods of Analysis of AOAC INTERNATIONAL. 2000. 17th edition, AOAC INTERNATIONAL, Gaithersburg, MD, USA.


Partial Characterization of Rice (*Oryza sativa* L.) cv. Khao Dawk Mali 105 as Affected by Accelerated-Aging Factors

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\(^3\)Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
\(^4\)Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok 10332, Thailand
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**ABSTRACT**

This study concerns the effect of accelerated-aging treatments on pasting properties, textural properties, solid loss, amylose content, cooked kernel elongation, color and the quantities of the key off-odor, n-hexanal, and the aroma-impact compound, 2-acetyl-1-pyrroline, of Thai Jasmine rice. Milled rice samples derived from freshly-harvested paddy with moisture contents of 13.4 and 16.6 percent wet basis were exposed to three designed sets of accelerated-aging conditions: 100°C for 60, 90 and 120 minutes, 110°C for 30 and 45 minutes, and 120°C for 15 and 30 minutes. Comparison between treated, untreated and naturally-aged samples revealed that accelerated-aging treatments enhanced the aging process of fresh rice samples, with the effect being significant in high-moisture-content rice and in higher temperature or longer exposure treated rice. The hardness and springiness of accelerated-aged cooked rice increased but its adhesiveness decreased. The accelerated-aged rice showed lower solid loss, higher yellowness, higher kernel elongation and pasting behavior similar to those of naturally-aged rice, though amylose content remained unchanged. The content of 2-acetyl-1-pyrroline and n-hexanal decreased in accelerated-aged rice, however, these were still higher than those of 6- to 12-month naturally-aged samples. The accelerated-aging technique designed in this study can be utilized for aging enhancement of Thai fragrant rice.

**Key words:** Aromatic rice, Accelerated aging, Physicochemical property, 2-acetyl-1-pyrroline, n-hexanal
INTRODUCTION

Demand for high quality fragrant rice has increased dramatically during the last decade. As one of the world’s biggest rice suppliers, Thailand has been alert to pay more attention to improving the quality of its fragrant rice products. Among the rice varieties Thailand exports, Khao Dawk Mali (KDML) 105, known as Thai Jasmine rice in international markets, is the most important variety. This is due to its unique aroma character, which is accepted by most Asian consumers, as well as consumers in the United States (Meullenet et al., 2001) and in some European countries. In addition to the pleasant aroma of some fragrant rice varieties, textural property is another major determinant factor for the majority of rice consumers. Most Asian populations prefer rice with harder and fluffier texture (Juliano, 1985). This explains the practice that KDML 105 rice for sale to Asian people is stored for a certain period of time to allow the formation of the preferred textural quality. However, the aromatic quality of rice, as measured by the amount of the impact aroma compound 2-acetyl-1-pyrroline (Buttery et al., 1982; 1988; Adams and De Kimpe, 2006), decreases during storage (Laksanalamai and Ilangantileke, 1993; Widjaja et al., 1996; Wongpornchai et al., 2004; Yoshihashi et al., 2005). Also, costs that result from longer and varied storage periods are added to the overall cost of rice. A technique called accelerated aging is a postharvest technology that has been advanced to lower storage and marketing costs.

Accelerated aging of freshly-harvested paddy, using wet or dry heat treatment with suitable grain moisture content, had been reported to improve some quality attributes that could be comparable to those of naturally-aged rice (Gujral and Kumar, 2003; Soponronnarit et al., 2008). Such practice on the paddy, however, resulted in lower head rice yield in the subsequent milling process. This was caused by fissures generated from dehydration of the incomplete gelatinized starch in the rice endosperm. Discoloration of the rice occurs due to the diffusion of husk and bran pigments into endosperm of paddy during moistening and heating steps, as occurred in parboiled rice (Lamberts et al., 2006). As husk is a barrier of moistening, heating and drying processes, accelerated aging of paddy requires more space, energy and time during processing.

An alternate accelerated-aging method, using milled rice, is proposed in this study. This method enhances aging by heating milled rice that is loaded in a closed system to prevent loss of water from its kernel. This can be an efficient process since it has several advantages over that using paddy. However, few studies have reported its effectiveness of improving physicochemical properties related to cooking quality, especially the aroma characteristic of fragrant rice. In this study, freshly-harvested KDML 105 milled rice samples with different moisture contents were subjected to a designed series of accelerated-aging treatments. Then, some physicochemical properties as well as quality parameters such as texture, color, solid loss and viscosity were characterized. Additionally, quantities of some active volatiles that have prominent effect on aroma quality of rice, which are 2-acetyl-1-pyrroline and n-hexanal, were determined.
MATERIALS AND METHODS

Sample preparation

The KDML 105 rice used in this study was cultivated in the growing season of 2005 at the Lampang Agricultural Research and Training Center, Rajamangala University of Technology Lanna, located in northern Thailand. The rice was harvested at maturity by hand, left to dry 2 to 3 days in the field and then threshed to paddy of approximately 14 percent moisture content. The freshly-harvested paddy sample was divided into two portions. One portion was stored as paddy in jute sacks under ambient conditions and designated as a naturally-aged sample. The other portion was prepared for accelerated-aging treatment. The paddy was de-hulled by a McGill sample sheller and the resulting brown rice was milled for 30 seconds in a friction-type miller operating with a 1.0 kg weight positioned at the end of a 25-cm mill lever arm. Head rice was separated from the broken kernel by a cylinder grader and used for subsequent treatments. The amylose content of the head rice sample was 17.59 percent (w/w). Protein (NX5.95) and lipid contents of the head rice as determined by AOAC (1999) standard methods were 7.64 and 0.88 percent, respectively.

Accelerated-aging treatments

Prior to accelerated-aging treatments, the head rice sample was divided into two portions by a Boerner divider (Seedburo Equipment Co., Chicago, Illinois). The first portion was allowed to equilibrate in room atmosphere and the other portion was adjusted to have high moisture content by placing the samples in sealed plastic boxes containing distilled water at room temperature for seven days. The moisture content of both sample portions was determined in triplicate on the seventh day by drying the samples in an oven at 103°C for 17 hours. The moisture content was 13.4 percent for ordinary rice grains and 16.6 percent based on wet basis for high-moisture-content rice grains. Processing of the accelerated-aging rice was done by sealing 370 grams of rice in aluminum containers. These containers were then exposed to temperatures of 100°C for 60, 90 and 120 minutes, to 110°C for 30 and 45 minutes and to 120°C for 15 and 30 minutes. This heat exposure was done in an automatic autoclave (SS-320, Tomy Seico Co. Ltd., Wako, Saitama, Japan). After exposure, the rice samples were left covered in the aluminum containers and cooled for 2 hours at 21°C. The rice samples were then poured into zippered plastic bags and kept at 4°C for further analyses.

Determination for pasting characteristics

Rice samples were ground to pass through a 0.5 mm screen (Cyclotec 1093 sample mill, Tecator, Hogenas, Sweden) and pasting characteristics of the flour samples were analyzed twice using a rapid visco analyzer (Model 4D, Newport Scientific, Warriewood, NSW, Australia). The flour samples, each weighing 3.00±0.01 g, this weight being adjusted based on a 12-percent moisture content, were placed in test canisters to which distilled water was added to each to make the weight of each 28.00±0.02 g. The samples were analyzed, as outlined by the AACC Approved Method 61-02 for the determination of pasting properties of
rice, with a rapid visco analyzer (AACC, 2000). Recorded analysis results were pasting temperature, peak viscosity, viscosity at 95°C after holding (trough), viscosity at 50°C (final viscosity), breakdown based on peak viscosity minus trough and setback based on final viscosity minus peak viscosity.

**Determination for textural properties of cooked rice**

Textural properties of cooked rice samples were determined in five replicates, using a bench-top TA-XTplus texture analyzer (Texture Technologies Corp., Scarsdale, New York). A two-cycle compression, force versus distance, was programmed and a 40-mm diameter cylindrical probe attached to a 50 kg load cell was used. The probe was set at 5 mm above the base platform of the instrument and was allowed to travel 4.9 mm, return and repeat at a test speed of 1 mm/sec. Rice samples of 250 g were cooked at a rice-to-water volume ratio of 1:1.25 in a 1.1-liter rice cooker (Sharp model KSH-111). This step was followed by a 10-minute warming period. Samples of the cooked rice were taken by a spoon and the top 1-cm layer of each was discarded. Ten unbroken kernels from each sample were immediately arranged in a single layer on the base platform and subjected to texture profile analysis. The resulting 2-cycle test curves were then analyzed using the Texture Exponent 32 software (Stable Micro Systems, Godalming, UK). The texture profile analysis parameters recorded were hardness, being the peak force of first compression in grams, adhesiveness, being the negative force to pull probes from samples in g mm, cohesiveness, being the ratio of area under second compression to area under first compression and springiness, the ratio of distance traveled by the probe on the two curves, being related to sample recovery after first compression. All of these parameters were determined in three replicates.

**Determination for solid loss, amylose content, kernel elongation and color**

Solid loss during cooking was determined by boiling 5.00 g of milled rice in a test tube containing 30 ml of distilled water for 15 minutes in a hot water bath of 99±1°C. The drained cooking water was oven-dried and weighed to determine the percent of solid loss. Amylose content was determined according to the method of Juliano et al. (1981). Kernel elongation was an average of 10 unbroken cooked rice kernels of samples prepared for determining textural properties. Rice sample color was measured using a Hunterlab color meter (ColorQuest® XE, Hunterlab, Reston, Virginia, USA), using the 1976 Commission Internationale de l’Eclairage L* a* and b* color system. Color parameters interpreted for L* and b* values describe the brightness and yellowness of samples, respectively. All of these characteristics were determined in three replicates.

**Analysis of 2-acetyl-1-pyrroline and n-hexanal**

The amounts of 2-acetyl-1-pyrroline (2AP) and n-hexanal, representing the impact aroma and off-odor compounds of the rice samples, were analyzed using the headspace-gas chromatography (HS-GC) method developed by Sriseadka et al., (2006). Milled rice samples were ground to pass through a 0.5 mm screen.
The resulting flour, weighing exactly 1.000 g was placed into a 20-ml headspace vial. An internal standard of 1 μL of 0.50 mg/ml 2,6-dimethylpyridine (DMP) in benzyl alcohol was added to the vial, which was then immediately sealed with a PTFE/silicone septum (Restek Corp., Bellefonte, Pennsylvania) and an aluminum cap. An Agilent Technologies (Wilmington, Delaware) gas chromatograph, model 6890N, equipped with headspace autosampler (Agilent Technologies model G1888) and a fused silica capillary column, HP-5, with a 5% phenyl-95% dimethylpolysiloxane 1.5 μm film thickness chemical coat and dimensions of 30 m × 0.53 mm i.d. (J&W Scientific, Folsom, California), was employed. Sample headspace vial was equilibrated at 120°C for 9 minutes in the autosampler before the rice headspace was transferred to the injection port of the GC. The GC condition was set as follows: the column temperature program started at 50°C and increased at a rate of 1°C /minute to 70°C, the injector and flame ionization detector temperatures were 230°C and 250°C, respectively. Purified helium was used as carrier gas at a flow rate of 7mL/minute. Concentrations of 2AP in the rice samples were determined by using a standard calibration curve. The relative amounts of n-hexanal were derived from the ratio of the peak areas of n-hexanal and DMP, which was added to the rice samples as an internal standard.

Statistical analysis

Data regarding physicochemical properties and aroma quality were statistically analyzed using analysis of variance (ANOVA) to determine the effect of grain moisture content, temperature and heating duration. Duncan’s multiple range test, $P<0.05$, was done to separate the means. Correlation coefficients (r) between rice quality parameters were calculated when appropriate.

RESULTS AND DISCUSSION

Physicochemical property parameters related to cooking and eating quality, such as pasting and textural properties, color parameters $L^*$ and $b^*$, the impact volatiles 2AP and n-hexanal and kernel elongation of the naturally-stored KDML 105 rice (NA sample), are shown in Table 1. These parameters changed as a function of storage time and the resulting changes corresponded with those reported previously (Widjaja et al., 1996; Perdon et al., 1997; Sowbhagya and Bhattacharya, 2001; Zhou et al., 2002; Wongpornchai et al., 2004; Yoshihashi et al., 2005). These values were used as references for comparison with those obtained from the AA treatments.

Pasting properties of the NA and AA rice, as measured from flour samples by rapid visco analyzer, are shown in Tables 1 and 2, respectively. The AA treatments altered the pasting behavior of fresh rice by causing a severe effect on high-moisture-content samples and on those samples being subjected to higher temperature and longer exposure duration. In general, pasting curves of AA rice were elevated except for both ordinary and high-moisture-content samples treated at 120°C for 30 minutes and for high-moisture-content samples treated at 100°C for 120 minutes, of which the peak viscosity decreased compared to that of fresh
Table 1. Pasting properties, textural properties, color parameters ($L^*$ and $b^*$), key volatile compounds and kernel elongation of KDML 105 rice during storage as paddy for up to 12 months at ambient temperature.

<table>
<thead>
<tr>
<th>Rice attributes</th>
<th>Storage time (months)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasting properties (cP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- peak viscosity</td>
<td>3335±32d</td>
<td>3659±68c</td>
<td>3846±24a</td>
<td>3766±12c</td>
<td>3630±12c</td>
<td>3305±23d</td>
<td>3271±21d</td>
<td></td>
</tr>
<tr>
<td>- trough</td>
<td>2308±147c</td>
<td>2440±40b</td>
<td>2601±60a</td>
<td>2633±43a</td>
<td>2574±63a</td>
<td>2227±20c</td>
<td>1936±42d</td>
<td></td>
</tr>
<tr>
<td>- final viscosity</td>
<td>3433±126e</td>
<td>3619±44d</td>
<td>3840±48c</td>
<td>4239±54a</td>
<td>4124±35b</td>
<td>3710±20d</td>
<td>3621±14d</td>
<td></td>
</tr>
<tr>
<td>- breakdown</td>
<td>1027±146c</td>
<td>1219±71ab</td>
<td>1245±64a</td>
<td>1133±49bc</td>
<td>1056±52c</td>
<td>1078±21c</td>
<td>1334±27a</td>
<td></td>
</tr>
<tr>
<td>- setback</td>
<td>98.0±125.38c</td>
<td>-40.0±99.24d</td>
<td>-5.5±40.83cd</td>
<td>473.2±44.73a</td>
<td>494.0±22.85a</td>
<td>405.1±7.78ab</td>
<td>350.0±8.45b</td>
<td></td>
</tr>
<tr>
<td>- pasting temperature (°C)</td>
<td>80.67±0.41d</td>
<td>83.00±0.44c</td>
<td>82.46±1.16c</td>
<td>84.52±0.15b</td>
<td>86.38±0.31a</td>
<td>85.98±0.15a</td>
<td>86.50±0.55b</td>
<td></td>
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<tr>
<td>Textural properties</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>- hardness (g) ($P^2&lt;0.075$)</td>
<td>14960±440ab</td>
<td>14853±297ab</td>
<td>14462±458b</td>
<td>14775±527ab</td>
<td>14915±558ab</td>
<td>15546±245a</td>
<td>15488±361a</td>
<td></td>
</tr>
<tr>
<td>- adhesiveness (g. mm)</td>
<td>647±28.51b</td>
<td>581±88.86b</td>
<td>473±66.10a</td>
<td>417±9.24a</td>
<td>401±29.64a</td>
<td>440±33.41a</td>
<td>436±80.32a</td>
<td></td>
</tr>
<tr>
<td>- springiness</td>
<td>0.191±0.020c</td>
<td>0.205±0.011bc</td>
<td>0.187±0.007c</td>
<td>0.213±0.013ab</td>
<td>0.224±0.009ab</td>
<td>0.207±0.008bc</td>
<td>0.231±0.007a</td>
<td></td>
</tr>
<tr>
<td>- cohesiveness</td>
<td>0.566±0.028</td>
<td>0.561±0.005</td>
<td>0.541±0.013</td>
<td>0.547±0.002</td>
<td>0.558±0.009</td>
<td>0.575±0.018</td>
<td>0.555±0.007</td>
<td></td>
</tr>
<tr>
<td>Color parameters</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>- brightness ($L^*$ value)</td>
<td>51.09±1.53ab</td>
<td>47.78±1.05d</td>
<td>48.53±0.67cd</td>
<td>50.22±1.07bc</td>
<td>52.47±1.55a</td>
<td>51.47±1.06ab</td>
<td>50.17±0.73bc</td>
<td></td>
</tr>
<tr>
<td>- yellowness ($b^*$ value)</td>
<td>7.00±0.11f</td>
<td>8.74±0.03de</td>
<td>9.36±0.25bc</td>
<td>9.44±0.25b</td>
<td>8.52±0.15</td>
<td>8.99±0.08cd</td>
<td>9.85±0.40a</td>
<td></td>
</tr>
<tr>
<td>Key volatile compounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 2-acetyl-1-pyrroline (ppm)</td>
<td>5.57±0.20a</td>
<td>4.49±0.16b</td>
<td>3.57±0.17c</td>
<td>2.75±0.14d</td>
<td>2.78±0.11d</td>
<td>2.80±0.11d</td>
<td>2.30±0.08e</td>
<td></td>
</tr>
<tr>
<td>- n-hexanal (area ratios of DMP)</td>
<td>0.45±0.05e</td>
<td>0.57±0.02d</td>
<td>0.91±0.09ab</td>
<td>0.84±0.08c</td>
<td>0.75±0.02c</td>
<td>0.22±0.009b</td>
<td>0.20±0.008c</td>
<td></td>
</tr>
<tr>
<td>- kernel elongation (mm)</td>
<td>9.87±0.12b</td>
<td>NA</td>
<td>NA</td>
<td>10.12±0.25b</td>
<td>NA</td>
<td>10.03±0.10b</td>
<td>10.78±0.24a</td>
<td></td>
</tr>
</tbody>
</table>

Means (±SD) followed by the same letters in a row are not significantly different ($P<0.05$).

This decrease of peak viscosity indicates the high impact on aging of these AA treatments. Pasting property parameters, such as pasting temperature, final viscosity and setback increased consistently with increasing exposure duration regardless of temperature levels. In contrast, peak viscosity, trough for high moisture content samples and breakdown had a decreasing trend after receiving higher temperature or longer duration treatments. This trend was similar to that of the naturally-aged samples and was in agreement with trends reported in literature (Perdon et al.,

Table 2. Rapid visco analyzer (RVA) viscosity parameters of flour from KDML 105 freshly-harvested milled rice samples as affected by accelerated-aging factors of grain moisture content, temperature and exposure duration.

<table>
<thead>
<tr>
<th>Grain moisture content (% wb)</th>
<th>Temperature -exposure duration (°C-min)</th>
<th>Pasting temp. (°C)</th>
<th>Peak viscosity</th>
<th>Trough</th>
<th>Final viscosity</th>
<th>Breakdown</th>
<th>Setback</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh rice</td>
<td>80.7±0.4h</td>
<td>3335±32f</td>
<td>2308±147bcd</td>
<td>3433±126j</td>
<td>1027±146g</td>
<td>98.0±125.4e</td>
<td></td>
</tr>
<tr>
<td>13.4</td>
<td>83.8±0.9g</td>
<td>3802±29bc</td>
<td>2400±125bcd</td>
<td>3670±115ghi</td>
<td>1402±103abc</td>
<td>131.5±96.2f</td>
<td></td>
</tr>
<tr>
<td>100-60</td>
<td>85.4±0.8f</td>
<td>4015±49a</td>
<td>2540±148ab</td>
<td>4021±138cd</td>
<td>1505±108a</td>
<td>-24.5±93.3f</td>
<td></td>
</tr>
<tr>
<td>100-90</td>
<td>88.2±0.1c</td>
<td>3616±81cde</td>
<td>2647±111a</td>
<td>4342±91a</td>
<td>969±77g</td>
<td>726.0±55.9c</td>
<td></td>
</tr>
<tr>
<td>110-30</td>
<td>84.6±0.7g</td>
<td>3714±142bcde</td>
<td>2431±204abc</td>
<td>3709±164ghi</td>
<td>1283±65bcd</td>
<td>-5.2±40.9f</td>
<td></td>
</tr>
<tr>
<td>110-45</td>
<td>85.5±0.4f</td>
<td>3821±18b</td>
<td>2390±95bcd</td>
<td>3821±96efg</td>
<td>1431±89a</td>
<td>0.3±80.2ef</td>
<td></td>
</tr>
<tr>
<td>120-15</td>
<td>83.9±0.2g</td>
<td>3619±54cde</td>
<td>2361±19bcd</td>
<td>3589±20hij</td>
<td>1258±72cde</td>
<td>-29.5±54.7ef</td>
<td></td>
</tr>
<tr>
<td>120-30</td>
<td>88.9±0.4bc</td>
<td>3101±275g</td>
<td>2511±192ab</td>
<td>3786±179fgh</td>
<td>321±51i</td>
<td>1295.8±107.7a</td>
<td></td>
</tr>
<tr>
<td>16.6</td>
<td>80.5±0.2f</td>
<td>3724±80bcde</td>
<td>2360±74bcd</td>
<td>3768±876gh</td>
<td>1364±24abc</td>
<td>44.7±62.6e</td>
<td></td>
</tr>
<tr>
<td>100-60</td>
<td>83.8±0.9g</td>
<td>3655±46bcde</td>
<td>2494±22ab</td>
<td>4086±15bcd</td>
<td>1161±34def</td>
<td>431.5±58.9d</td>
<td></td>
</tr>
<tr>
<td>100-90</td>
<td>89.7±0.3d</td>
<td>3655±46bcde</td>
<td>2494±22ab</td>
<td>4086±15bcd</td>
<td>1161±34def</td>
<td>431.5±58.9d</td>
<td></td>
</tr>
<tr>
<td>110-30</td>
<td>85.7±0.2f</td>
<td>3739±10bcd</td>
<td>2358±137bcd</td>
<td>3730±106gh</td>
<td>1381±147abc</td>
<td>-9.0±116.2e</td>
<td></td>
</tr>
<tr>
<td>110-45</td>
<td>86.4±0.3de</td>
<td>3562±03d</td>
<td>2439±132abc</td>
<td>3952±147df</td>
<td>1123±70f</td>
<td>389.8±86.4d</td>
<td></td>
</tr>
<tr>
<td>120-15</td>
<td>85.4±0.4f</td>
<td>3547±81e</td>
<td>2222±94cd</td>
<td>3506±80ij</td>
<td>1325±67bc</td>
<td>-41.3±70.3ef</td>
<td></td>
</tr>
<tr>
<td>120-30</td>
<td>90.4±0.2a</td>
<td>2490±118i</td>
<td>2169±90d</td>
<td>3786±179gh</td>
<td>321±51i</td>
<td>1295.8±107.7a</td>
<td></td>
</tr>
</tbody>
</table>

Means (±SD) followed by the same letters in a column are not significantly different ($P<0.05$).
1997; Sowbhagya and Bhattacharya, 2001; Zhou et al., 2003; Soponronnarit et al., 2008) in that peak viscosity, trough and breakdown increased during the first few months of storage and then declined, or even disappeared, during prolonged storage. This change reflected the complexity of the aging process. However, results from this study imply that aging effects taking place in both AA and NA rice are probably based on the same phenomenon.

Changes in pasting properties during aging have been reported to be associated with changes in starch granule components (Martin and Fitzgerald, 2002; Zhou et al., 2002; 2003; Fitzgerald et al., 2003), with protein oxidation being a key process. This change in starch granule components decreased the hydrophilic property of the surface protein of the rice starch granule, leading to the limitation of its hydration and swelling capacity (Zhou et al., 2003). As the results from this study, changes of pasting properties in NA and AA rice samples can be explained that both NA and AA processes would decrease the hydration property of the rice starch granule and consequently increase its rigidity. These changes occurred continuously in rice samples during natural storage and with enhanced rate in the AA treatments. The increase in pasting temperature of the NA and AA viscosogram confirmed the reduction in starch granule hydrophilic properties. The increase of peak viscosity observed in the first 2 to 4 months of natural storage and in the less-severe AA condition was attributed to the increase in rigidity of granules that could withstand rupture during pasting. Lower amylase activity due to storage (Dhaliwal et al., 1991) or denaturing of the enzyme by heat in this study would also contribute to this phenomenon. These aged granules, as compared to fresh-rice granules, could be more resistant to shearing stress and could swell to a larger size in the limited amount of hot water during the rapid visco analyzer measurement. With increased storage time or the increasing intensity of AA, the rigidity of the starch granules continued to increase and, thus, could limit swelling and disintegration of starch granules, resulting in lower peak viscosity values. The progressive increases in final viscosity and setback reflected the degree of retrogradation increase in rice samples after AA treatments, which were similar to those that occurred in the NA rice stored for 6 to 12 months, as shown in Table 1.

Textural properties of cooked rice prepared from AA rice samples were investigated through texture profile analysis and the results are presented in Table 3. These AA treatments significantly changed the fresh rice texture profile analysis attributes of hardness, adhesiveness and springiness, an exception being cohesiveness. Cooked milled rice exposed to higher temperature with long durations of 120°C for 30 minutes and 100°C for 120 minutes had significantly higher hardness and springiness, but lower adhesiveness than fresh rice and those rice samples obtained from the lower temperature and shorter duration treatments. The effects of AA treatment were more pronounced in high-moisture-content grains than in ordinary-moisture content-grains. This is seen in the greater hardness of the high-moisture-content samples under 100°C for 90-minute and 110°C for 45-minute treatments. Hardness increased by 9.2 percent and adhesiveness decreased by 60.2 percent in the most severe AA conditioned sample, this being
high-moisture-content milled rice exposed at 120°C for 30 minutes, as compared with those of fresh rice. These results are in accordance with those reported by Gujral and Kumer (2003) in that hardness, springiness and cohesiveness increased and adhesiveness decreased to different degrees during the accelerated aging of freshly-harvested paddy with varying moisture content by steaming. The small increase of hardness is probably because of the soft texture of the KDML 105 rice variety used in this study. It is well-known that KDML 105 rice variety is low in amylase content and its cooked kernel has a soft texture. The AA method used in this study, therefore, showed the potential of modifying the textural properties of freshly-harvested KDML 105 rice to those of aged rice without changing much of its typical soft texture.

Table 3. Texture profile analysis attributes of cooked freshly-harvested KDML 105 rice as affected by accelerated-aging factors of grain moisture content, temperature and exposure duration.

<table>
<thead>
<tr>
<th>Grain moisture content (% wb)</th>
<th>Temperature-exposure duration (°C-min)</th>
<th>Texture profile analysis attributes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hardness (g)</td>
</tr>
<tr>
<td>Fresh rice</td>
<td></td>
<td>14960±414e</td>
</tr>
<tr>
<td>13.4</td>
<td>100-60</td>
<td>15346±157de</td>
</tr>
<tr>
<td></td>
<td>100-90</td>
<td>15506±336de</td>
</tr>
<tr>
<td></td>
<td>100-120</td>
<td>16138±317abc</td>
</tr>
<tr>
<td></td>
<td>110-30</td>
<td>14908±541e</td>
</tr>
<tr>
<td></td>
<td>110-45</td>
<td>15470±461de</td>
</tr>
<tr>
<td></td>
<td>120-15</td>
<td>15308±118de</td>
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<td>120-30</td>
<td>16237±474ab</td>
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<td>16.6</td>
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<td></td>
<td>100-90</td>
<td>15706±285bcd</td>
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<td></td>
<td>100-120</td>
<td>16272±234ab</td>
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<tr>
<td></td>
<td>110-30</td>
<td>15546±173cde</td>
</tr>
<tr>
<td></td>
<td>110-45</td>
<td>15841±338abcd</td>
</tr>
<tr>
<td></td>
<td>120-15</td>
<td>15646±275de</td>
</tr>
<tr>
<td></td>
<td>120-30</td>
<td>16339±270a</td>
</tr>
</tbody>
</table>

Means(±SD) followed by the same letters in a column are not significantly different (P<0.05).

Effects of grain moisture content, temperature level and exposure duration on solid loss, elongation of cooked kernels, amylase content and color parameters of L* and b* are shown in Table 4. Solid loss during cooking was substantially decreased in rice exposed to AA treatments of higher temperature and longer time. This decrease led to the reduction in adhesiveness of the AA cooked rice, as indicated by the association between adhesiveness and solid loss, r = 0.70, P<0.01. This result was in line with the work conducted by Gujral and Kumer (2003) who reported that loss of solid and adhesiveness of cooked rice decreased
after paddy had received accelerated-aging treatments. Reductions of solid loss in ambient storage and accelerated-aged KDML 105 paddy were also reported by Soponronnarit et al., (2008). They stated that solid loss was reduced from 2.81 percent in unheated reference fresh rice to 1.78 percent in the most heated sample, in which the heating temperature was 150°C, grain moisture content was 33.2 percent dry basis and tempering time was 120 minutes. This reduction was almost equivalent to the 1.84 percent reduction noted in 6-month stored natural rice. The reduction was attributed to the strengthening of cell walls and to the complex formation between free fatty acid and amylose molecules, which could lower grain swelling and starch solubility during cooking. The heat levels used in this study for AA of milled rice were sufficient to enhance the rate of aging and to cause more-organized starch granules. These aged granules became less susceptible to disintegration which consequently decreased solid loss during cooking. Better integrity of NA and AA aged rice grains was confirmed by both NA and AA kernel elongation data shown in Tables 1 and 4. Hence, with less disintegration, cooked kernels of AA and 12-month NA samples were significantly longer than those of fresh rice. After AA treatments, amylose content in rice samples remained unchanged and, thus, could not account for any differences in solid loss or in textural and pasting properties of the samples.

Table 4. Solid loss, amylose content, kernel elongation and color parameters \(L^*\) and \(b^*\) of KDML 105 freshly-harvested milled rice as affected by accelerated-aging factors of grain moisture content, temperature and exposure duration.

<table>
<thead>
<tr>
<th>Grain moisture content (% wb)</th>
<th>Temperature-exposure duration (°C-min)</th>
<th>Solid loss (%)</th>
<th>Amylose content (%)</th>
<th>Kernel elongation (mm)</th>
<th>(L^*) value (brightness)</th>
<th>(b^*) value (yellowness)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh rice</td>
<td></td>
<td>6.21±1.38a</td>
<td>17.59±1.40</td>
<td>9.87±0.12f</td>
<td>51.09±1.54</td>
<td>7.00±0.11gh</td>
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<td>13.4</td>
<td>100-60</td>
<td>5.56±0.83abc</td>
<td>17.11±1.09</td>
<td>10.34±0.15cde</td>
<td>52.96±1.38</td>
<td>7.72±0.36defg</td>
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<td></td>
<td>100-90</td>
<td>4.81±0.37abcede</td>
<td>17.11±1.15</td>
<td>10.61±0.34bcd</td>
<td>53.80±1.29</td>
<td>8.15±0.68cd</td>
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<td>3.28±0.32cde</td>
<td>16.66±0.80</td>
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<td>52.87±0.96</td>
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<tr>
<td></td>
<td>110-30</td>
<td>6.05±0.13a</td>
<td>17.12±1.51</td>
<td>10.18±0.12def</td>
<td>51.83±2.60</td>
<td>7.53±0.27defgh</td>
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<td>5.79±1.55a</td>
<td>16.73±0.88</td>
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<td>52.97±1.14</td>
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<td>17.43±1.03</td>
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<td>7.22±0.06fg</td>
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<td>10.94±0.16ab</td>
<td>53.27±0.40</td>
<td>9.10±0.20b</td>
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<td>5.66±1.69ab</td>
<td>17.46±0.82</td>
<td>10.70±0.18bc</td>
<td>51.49±1.91</td>
<td>7.30±0.59efgh</td>
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<tr>
<td></td>
<td>100-90</td>
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<td>52.05±0.78</td>
<td>7.82±0.09def</td>
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<td>8.78±0.15bc</td>
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<tr>
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<tr>
<td></td>
<td>110-45</td>
<td>5.25±1.09abcd</td>
<td>17.70±1.21</td>
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<td>50.70±2.06</td>
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<td></td>
<td>120-30</td>
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<td>11.09±0.40ab</td>
<td>50.57±1.68</td>
<td>9.82±1.07a</td>
</tr>
</tbody>
</table>

Means (±SD) followed by the same letters in a column are not significantly different (P<0.05).
Yellowness, the $b^*$ value, of AA milled rice increased with increasing temperature, exposure duration and grain moisture content. The $b^*$ value of the high-moisture-content fresh rice changed from 7.01 to a high value of 9.82 in the 120°C 30-minute treatment. Although this increase in yellowness was statistically significant, the $b^*$ values were only in acceptable ranges as regards to the reference $b^*$ value of the 12-month naturally-aged sample indicated in Table 1. The increase in yellow color was attributed to the Maillard reaction taking place in the AA process. The AA technique did not affect brightness, the $L^*$ value, of the milled rice samples. The $L^*$ values of these milled samples ranged from 50.29 to 53.80 and were not significantly different from the $L^*$ value of 51.09 of fresh rice. These results indicate the effectiveness of the AA technique in changing freshly-harvested rice to aged rice without altering much of its color.

Quantities of the aroma-impact compound, 2AP, which remained in the KDML 105 grain samples after AA processes, were determined in order to assess the effect of each of AA treatment on rice aroma quality. Grain moisture contents, temperature levels and exposure durations could affect the amount of 2AP in rice samples, as shown in Figure 1A. Regardless of grain moisture content, a decrease in 2AP content was observed when the exposure duration was prolonged. The 2AP content of NA rice decreased dramatically from 5.57 ppm at the beginning of storage to 2.30 ppm in 12-month stored samples (Table 1). This 2.30-ppm value was lower than those observed when the most severe AA condition was applied to rice samples. This fact suggests that rice aging can be accelerated to obtain a desired textural property while still maintaining high aroma quality in terms of 2AP content.

Relative amounts of the key off-odor compound, n-hexanal, generated during the AA process or in the period of natural storage, were also determined in terms of the area ratios of $n$-hexanal and DMP. After AA treatments, high-moisture-content grains showed lower amounts of $n$-hexanal compared to those of fresh and low moisture content grain samples (Figure 1). At a given temperature level, the amount of $n$-hexanal tended to be lower with a longer exposure duration, though the effects of temperature levels and exposure durations were not significantly different at $P<0.05$ level, except for the high value observed in the low-moisture-content sample heated at 120°C for 15 minutes.

This result suggests that a higher temperature and a longer exposure time during the AA process can accelerate volatilization of highly-volatile compounds, including $n$-hexanal, from the rice samples, leaving these rice grains with lower levels of $n$-hexanal and other lipid breakdown products. Thus, the highest content of $n$-hexanal in low moisture content sample heated at 120°C for 15 minutes may be attributed to insufficient heating time. For NA samples, the content of $n$-hexanal increased with increasing storage time from the initial value of 0.45 to 0.99 in 12-month stored samples (Table 1). The $n$-hexanal content of 4- to 12-month aged samples was almost three times higher than the average $n$-hexanal content of the AA samples, in which the degradation of lipids during storage was limited. Thus, this study showed that aged rice produced from this modified AA process had low amounts of the prime off-odor compound, $n$-hexanal, and suggests the
advantage and usefulness of the AA process technique.

![Graph A](image1.png)

**Figure 1.** Quantity of 2-acetyl-1-pyrroline (A) and area ratio of $n$-hexanal to DMP (B) of KDML 105 freshly-harvested milled rice as affected by accelerated-aging factors of grain moisture content, temperature and exposure duration.

**CONCLUSION**

This study showed that physicochemical properties related to cooking and eating quality of freshly-harvested Thai Jasmine milled rice could be altered to the characteristics qualitatively identical to those of naturally-aged rice by employing the AA technique. Fresh aromatic milled rice can be aged mildly, moderately or highly within a short time, depending on the level of the three factors, i.e., moisture content, temperature and duration, used in the AA operation. The technique, therefore, has proven to have a high potential to rapidly modify freshly-harvested rice to be rice of desirable cooking and eating properties while still maintaining aroma quality.
ACKNOWLEDGEMENTS

The authors would like to acknowledge the Postharvest Technology Research Institute, Postharvest Technology Innovation Center, Chiang Mai University, for financial and laboratory facility support, and the Center of Excellence for Innovation in Chemistry, Commission on Higher Education, Ministry of Education, for its support in lending the HS-GC instrument. Our special thanks are given to Mr. Tinakorn Sriseadka for his assistance in 2AP analysis.

REFERENCES


none
Contribution of Heterotrophic Respiration to Total Soil Respiration in a Wheat Field

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ABSTRACT

The contribution of soil respiration needs to be understood to evaluate the implications of environmental change on soil carbon cycling and sequestration. The response of soil respiration to varying environmental factors was studied in a wheat field. The continuous soil gradient method combined with the trench method was used to (1) determine the temporal variation of total soil respiration (Rs) and heterotrophic respiration (Rh) and (2) investigate the relative effect of soil temperature (Ts) and soil water content (Ws) which control soil respiration. The result showed that temporal variations of soil respiration were dominantly controlled by Ts during the days. The variation in Rs and Rh showed a similar pattern of seasonal change in Ts (0.69 to 4.17 μmol m⁻²s⁻¹ and 0.45 to 2.95 μmol m⁻²s⁻¹, respectively). Rh ranged from 36% - 86% of Rs. The Rs was limited by Ws while Ts played as a secondary role; Rh, however, appeared to be correlated with both Ts and Ws. These results suggested that the factors controlling the variation in soil respiration differed between Rh and Rs. Additionally, two-variable equations could be better used to model the relationships of soil respiration to both Ts and Ws together, with the R² ranging from 0.53 to 0.83.

Key words: Heterotrophic respiration, Soil respiration, Soil temperature, Soil water content

INTRODUCTION

Carbon dioxide (CO₂) emission from the soils is an important component of the global carbon (C) cycle and has been shown to play a role in global warming. Extensive evidence suggests that this is associated with the increasing atmospheric CO₂ concentration (Schlesinger and Andrews, 2000). Soil respiration typically accounts for more than three-quarters of the CO₂ released through ecosystem respiration (Law et al., 2001) and is primarily controlled by temperature and soil moisture (Lloyd and Taylor, 1994; Davidson et al., 1998; Fang and Moncrieff, 1999; Jassal et al., 2008). It is thought that even a small increase in global
warming leading to a higher soil temperature is likely to increase soil CO$_2$ emissions through increased respiration which, in turn, are thought to lead to an appreciable increase in atmospheric CO$_2$ concentration. Therefore, it is important to obtain a good estimates of soil respiration and its relation to environmental controls.

The total respiration from the soil surface usually refers to soil respiration which mainly includes respiration from plant roots (autotrophic respiration) and microorganisms (heterotrophic respiration). Since autotrophic and heterotrophic respiration react differently to change in environmental conditions, it is crucial to get more insight into both components of soil respiration. However, the separation of heterotrophic respiration from total soil respiration under a field conditions remains difficulty since there are no effective, non-intrusive methods to separate them without disturbing the root and microbial organisms activities (Buchmann, 2000; Wang and Yang, 2007). In addition, data that might otherwise have been obtained from the greenhouse or laboratories are not likely faithfully reflect natural outdoor soil-atmosphere conditions. Three primary methods have generally been used to separate heterotrophic respiration from total soil respiration, i.e. (1) the integration of components, (2) the root exclusion method (trenching method), and (3) the use of stable isotopes (Hanson et al., 2000). The trenching method calculates the difference between CO$_2$ emission rates from soil volumes in which roots are either present or excluded to determine heterotrophic respiration. This method is relatively simple and can provide realistic estimates of heterotrophic respiration. Although the trenching method has been used in forest ecosystems and grassland ecosystems (Lee et al., 2003; Tang et al., 2005; Ngao et al., 2007) but it is still unknown whether this method is suitable in the measurements of heterotrophic respiration in agricultural fields. Thus, the bias introduced by using the trenching method should be quantified in order to accurately estimate heterotrophic respiration.

Numerous efforts have been made to understand the mechanisms behind the variation of soil respiration and empirical models have been developed to predict soil respiration using biophysical factors such as soil temperature, soil water content and their interaction (Lloyd and Taylor, 1994; Davidson et al., 1998; Tang et al., 2005; Vincent et al., 2006). However, none of these models appears to be consistently better than the others. In addition, models or equations have seldom been validated against independent data sets. Generally, soil respiration is related to many processes such as photosynthesis, root respiration, organic matter decomposition and microbial activity (Bunnell et al., 1997) and these processes are influenced by multiple biophysical factors. Therefore, root and heterotrophic respirations may respond and adapt to environmental variables (soil temperature and soil water content) differently and thus lead to different carbon flux patterns in a scenario of global climatic warming. The ability to separate soil respiration is thus essential to understand below-ground C processes and the dynamic processes and environmental controlling-factors of these components in agricultural soils have yet to be investigated.

In this study, we used the trenching plot combined with the soil CO$_2$ gradient method to determine heterotrophic respiration and total soil respiration in a wheat
field. The objectives of this paper were to (1) determine the temporal variation of total soil respiration and heterotrophic respiration and (2) investigate the relative effect of soil temperature and soil water content which control soil respiration.

MATERIALS AND METHODS

Site description

The experiment was conducted in a 6 ha of non-irrigated wheat field at the Southwest Georgia’s Research and Education Center, Plains, Georgia, USA, (32.050° N, 84.367° W; 156 m elevation) during November 2006 to May 2007. The field was relatively flat in our sampling area. Wheat (Triticum aestivum L., var. Ag South 2000) was planted on November 15, 2006 and harvested on May 14, 2007 with a yield of 5,043 kg ha⁻¹. The soil was ploughed for land preparation prior to sowing. The sowing density of winter wheat was 56 kg per ha at a 0.06 m spacing. Basal fertilizer of N, P₂O₅, K₂O (4-22-6) was applied at 448 kg ha⁻¹ during planting and 56 kg ha⁻¹ of urea was applied before heading. The soil type was relatively uniform and dominated by sandy clay loam. The soil for planting wheat was composed of 52% of sand, 20% of silt and 28% of clay with a bulk density of 1.03 g cm⁻³ and 2.24% of organic matter. The crop was protected against pests and dioceses throughout the study.

Soil respiration measurements

Soil respiration was measured by using soil CO₂ gradient measurement systems during the period of February to May 2007. Soil respiration was also measured at two locations, i.e., inside a trenched plot and an untrenched plot. We created open space and established a small plot of 3 m x 3 m for the trenching method. We dug a trench 0.40 m deep and 1.20 m wide around the plot. After lining the trench with a polyethylene sheet, we put the soil back into the trench plot according to its original soil profiles while minimizing any disturbance. The trench cut down most live roots that extended into the plot. The barrier sheets were installed to inhibit future root growth. The trenched plot was then kept free of any vegetation by periodic manual removal. Thus, we assumed that there were no root influences within this plot. The untrenched plot was installed at one location and at a lateral distance of 3 m away from the center of the trenched plot. Thus, we also assumed that the trenched and untrenched plots were installed in a homogenous location.

In this study, total soil respiration (Rs) in the untrenched plot is defined as the combined root respiration of living root tissues and the respiration of symbiotic mycorrhizal fungi and associated microorganisms. Heterotrophic respiration (Rh) in the trenched plot is defined as the respiration of soil microorganisms and microorganisms not directly under the influence of the live root system.

All plots were installed with solid-state infrared gas analyzers (GMP343, Vaisala Inc., Finland) to continuously monitor soil CO₂ concentration profiles by burying two sensors at 4 and 8 cm soil depths during the vegetation period in the center of the trenched plot and in the soil beneath a wheat canopy in the untrenched
plot. The probe was 0.18 m in length and 0.055 m in diameter. Before installation, the sensors were covered with a sintered PTFE (polytetrafluoroethylene) filter and a cap made of POM (polyoxymethylene) with a diffusion slot enabling gas exchange between the soil and the probe and protecting the probe from water. The sensor’s dynamic range is 0-5,000 μmol mol⁻¹. The technical specification indicated that the accuracy of the CO₂ sensors is ± 5 ppm plus 2% of reading. The sensors were logged continuously and data were stored as 5-min averages in a datalogger (CR1000, Campbell Scientific Inc., Logan, UT). The sensors were installed in a horizontal face of a soil pit excavated at the site, keeping the different soil layers separated (Fig. 1). Then, soil layers were placed back in the same order to minimize the disturbance. The gradient measurement was applied to Fick’s gradient diffusion equation to calculate the CO₂ efflux from the soil:

$$F_z = -D_s \frac{dC}{dz}$$

(1)

where $F_z$ is the soil respiration, $D_s$ is the gaseous CO₂ diffusion coefficient in the soil that varies with soil, $C$ is the CO₂ mole concentration at a certain depth of the soil, and $z$ is the depth. For flux determination, the gradient is approximated by discrete differences $ΔC$ and $Δz$.

Diffusivity was computed with the Moldrup model (Moldrup et al., 2000)

$$\frac{D_s}{D_a} = \frac{\varepsilon^{2.5}}{\phi}$$

(2)

where $D_a$ is the CO₂ diffusion coefficient in the free air, $\varepsilon$ is the volumetric air content (air-filled porosity), $\phi$ the porosity or sum of the volumetric air content $\varepsilon$ and the volumetric water content ($W_s$).

Figure 1. A schematic presentation of the system for measuring soil CO₂ profile using solid-state CO₂ sensors (left) and trenching method (right).
Measurements of environmental factors

In tandem with soil respiration measurements, soil temperature was measured using thermocouples (type E, Omega Engineering, Inc, CT.) at depths of 4, 8, 12 and 30 cm near the CO$_2$ concentration sensors but at a lateral distance of 10 cm away from the probe. Volumetric soil water content was measured at depth of 0-4, 4-8 and 8-30 cm at the same location using time-domain reflectometry probes (CS616, Campbell Scientific Inc., Logan, UT). The CO$_2$ concentration, soil temperature and the data of the profile of volumetric soil water content were stored as 5-min average in a datalogger (CR1000, Campbell Scientific Inc., Logan, UT).

Half-hourly cumulative rainfall was measured above the canopy with a tipping bucket rain gauge with a resolution of 0.1 mm (TE525, Campbell Scientific Inc., Logan, UT). The 12 soil samples (0-15 cm depth) were collected using a soil corer. The soil samples were weighed, dried at 105°C for at least 48 hr and then re-weighed to calculate total soil porosity.

Data analysis

Linear and non-linear regression analyses were used to examine the relationships between soil respiration and environmental variables. Generally, soil temperature (Ts) and soil moisture (Ws) are considered to be the most influential environmental factors controlling soil respiration. Linear and non-linear regressions were performed to fit a simple empirical model to the daily soil CO$_2$ efflux mean data:

\[
F_s(T_s) = ae^{bT_s} \quad \text{(Lloyd and Taylor, 1994; Davidson et al., 1998)} \\
F_s(W_s) = a + bW_s + cW_s^2 \quad \text{(Qi and Xu, 2001)} \\
F_s(T_s, W_s) = ae^{bT_s}e^{cW_s + dW_s^2} \quad \text{(Tang and Baldocchi, 2005)}
\]

where $F_s$ is soil CO$_2$ efflux (µmol m$^{-2}$s$^{-1}$), Ts is the soil temperature (°C), Ws is the volumetric soil water content (m$^3$m$^{-3}$) and $a$, $b$, $c$ and $d$ are coefficients estimated by non-linear regression. Parameter $a$ from Equation 3 denotes the reference soil respiration at 0 °C and $b$ provides an estimate of the $Q_{10}$ coefficient (dependence of soil respiration on soil temperature). All statistical analyses were performed using Origins package, Version 7 (Origins Cooperation, Massachusetts, USA). Unless otherwise stated, significant differences of all statistical tests were evaluated at the level $\alpha = 0.05$.

RESULTS AND DISCUSSION

Diurnal and seasonal variations of soil respiration

Diurnal variations in soil respiration were highly associated with variation of soil temperature at 8 cm depth (Fig. 2) during the growing season. Diurnal soil water content at all depths changes were small on the days when rainfall did not
occur, indicating that soil water content was not a strong predictor of diurnal soil respiration patterns. In the untrenched plot, total soil respiration (Rs) followed the increasing trend of soil temperature in the morning and then decreased slightly when soil temperature decreased in the afternoon. Rs reached the peak values between 12:00-13:00 h. In contrast, heterotrophic respiration (Rh) was highest at 18:00 h, 2 h later than soil temperature at 8 cm depth and lowest at 11:00 h during a daytime (Fig. 2). Parkin and Kaspar (2003) reported that the CO$_2$ flux increased in response to soil warming in the morning and decreased when soil temperature started to cool, which is consistent with our soil respiration results from the trenched plot. It indicates that the diurnal variations in Rh closely resembled those in soil temperature. The mechanistic explanation of diurnal Rs in the untrenched plot is yet unclear. The effect may be due to a lag in production of CO$_2$ in the soil regulated by photosynthesis (Liu et al., 2006) or changes in photosynthate allocation to roots (Högberg et al., 2001; Liang et al., 2004).

Figure 2. Diurnal patterns of soil respiration and soil temperature at a depth of 8 cm in the untrenched and trenched plots. Open circles, increasing temperatures during the day.
The seasonal evolutions of the soil respiration components are presented in Fig. 3. Daily total soil respiration (Rs) and heterotrophic respiration (Rh) changed from 0.69 to 4.17 µmol m\(^{-2}\)s\(^{-1}\) and from 0.45 to 2.95 µmol m\(^{-2}\)s\(^{-1}\), respectively. These results are consistent with the previous reports from many croplands under different conditions (Lee and Jose, 2003; Han et al., 2006; Shi et al., 2006). The pattern of seasonal change in Rh in the trenched plot was similar to Rs in the untrenched plot during the day of year (DOY) 67-90. This may be attributed to the differences in root respiration and their exudates within the trenched plot. Soil temperature also showed the same pronounced seasonal pattern as the soil respiration. In contrast, soil water content at 4-8 cm depth showed a different pattern from soil temperature and soil respiration. Similar results have been reported by Xu and Qi (2001) and Han et al., (2006), suggesting that soil temperature was the primary factor controlling seasonal soil respiration.

![Figure 3](Figure 3. Seasonal variation of soil respiration in relation to soil temperature at 8 cm depth, volumetric soil water content at 4-8 cm depth and rainfall in the untrenched and trenched plots.)
Soil respiration and its correlation with soil temperature and soil moisture

By plotting soil respiration with soil temperature and soil water content at different depths, we found the correlation to be highest at the depth of 8 cm and 4-8 cm, respectively. This result indicated that soil temperature and soil water content at this depth were suitable to study the relationship between soil respiration and environmental factor. Table 1 summarizes the coefficients of determination and best single- and multiple-factor models obtained from evaluating the influences of the soil temperature and soil water content factors on the soil respiration. For the untrenched plot, the Rs showed a highly positive correlation with soil water content and the soil water content explained 58% variability in the Rs. For the trenched plot, 65% variability in the Rh during DOY 67-90 could be ascribed to the variability in the soil water content while 83% variability in the Rh during DOY 91-116 could be ascribed to the total variability in both soil temperature and soil water content.

Table 1. Parameters estimated for the models of soil respiration from the untrenched (Rs) and trenched (Rh) plots against soil temperature ($T_s$, °C) at 8 cm depth and soil water content ($W_s$, m$^3$ m$^{-3}$) at 4-8 cm depth.

<table>
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<tr>
<th>Environmental factors</th>
<th>a*</th>
<th>b*</th>
<th>c*</th>
<th>d*</th>
<th>R$^2$</th>
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<td></td>
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<tr>
<td>$W_s &lt; 0.13$</td>
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<td>-</td>
<td>0.41</td>
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<td>$0.13 &lt; W_s &lt; 0.16$</td>
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<td>0.07</td>
<td>-</td>
<td>-</td>
<td>0.55</td>
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<tr>
<td>$W_s &gt; 0.16$</td>
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<td>0.23</td>
<td>-</td>
<td>-</td>
<td>0.59</td>
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<td>2. $W_s$</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>DOY 67-116</td>
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<td>482.08</td>
<td>-1,600.48</td>
<td>-</td>
<td>0.58</td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>DOY 67-116</td>
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<td>0.06</td>
<td>140.86</td>
<td>-444.18</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>Models for the trenched plot (Rh)</strong></td>
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*a, b, c, d are significant coefficients ($\alpha < 0.05$). R$^2$ stands for determination coefficient.

We used simultaneously-measured of soil respiration to compare with the estimated soil respiration data. Three empirical models that predicted soil respiration were selected and fitted against the measurement of soil respiration data (Fig. 4a-c). The results show that the estimated of soil respiration data correlated well with the measured of soil respiration. About 76% and 87% of measured soil respiration was explained by the $F_s(\theta_s)$ and $F_s(T_s, \theta_s)$ equation in the untrenched and trenched plots, respectively. This result agrees with the finding of many researchers that the soil respiration are generally predicted by soil temperature (Lloyd and Taylor, 1994; Davidson et al., 1998; Xu and Qi, 2001; Han et al.,
2006), soil water content alone (Keith et al., 1997; Epron et al., 2004), or both (Bunnell et al., 1977; Mielnick and Dugas, 1999; Tang et al., 2005). In contrast to the single-factor model above, the $R^2$ of the multiple-factor model increased (Fig. 4b-c), therefore, the application of multiple-factor model was better than a single-factor model in predicting soil respiration.

Figure 4. Comparison of measured and modeled soil respiration in the untrenched and trenched plots: function of soil water content, $F_s(W_s)$ in the untrenched plot (a) and function of soil temperature, $F_s(T_s)$ and function of soil temperature and soil water content $F_s(T_s,W_s)$ in the trenched plot (b-c).

Effects of trenching plot on the measurements of heterotrophic respiration and environmental factors

The results show that trenching can modify soil environmental conditions. The plot trenching tends to increase in both $T_s$ and $W_s$ (Fig 2-3) leading to a significant difference in $T_s$ and $W_s$ between the untrenched and trenched plots. It was found that heterotrophic respiration (Rh) was underestimated in this study. This is likely an artifact of the experimental design, as the trenched plot's was
higher in temperatures which are likely to be an artifact resulting from an imperfect technique: (1) it is virtually impossible to prevent any soil disturbance by trenching the plot and (2) the radiation load over that plot is vastly different from that of the untrenched plot, making a true separation of the respiration components rife with uncertainties pertaining to the role of the higher temperature in the dataset. Another reason for obtaining the lower rates of Rh from the trenched plot soil could be the depletion of labile carbon. Since the trenched plot did not receive the labile carbon from the plant roots, its might have become depleted of the labile carbon compared to the untrenched plot. This could explain the lower rate of Rh that obtained from the trenched plot (Jiang et al., 2005; Ngao et al., 2007).

CONCLUSION

The present study sought to separate the contribution of heterotrophic respiration from the total soil respiration using a trenching method. Results suggest that total soil respiration (the untrenched plot) was more sensitive to soil water content than soil temperature. However, heterotrophic respiration (the trenched plot) was controlled by both soil temperature and soil water content, but soil temperature appeared to be a more important variable. Moreover, the seasonal variation in soil respiration can be predicted by the combination of soil temperature and soil water content in our field. Based on the multivariate regression analysis, the bi-variable model was better fitted well with the observed data and explained approximately 83% accounted of the total variation in daily soil respiration. By using of the trenching method for the purpose of separating heterotrophic respiration from the total soil respiration in agricultural soils should be carefully considered as it perturbs the soils and thus alters both soil water content and temperature, rendering any robust distinction of the role of heterotrophic and autotrophic respiration measurements. Results from the present experiment suggest that the characterization of the partitioning of total soil CO₂ emissions between autotrophic and heterotrophic respiration can be achieved provided that (1) smaller-area trenched plots should be used to reduce the radiation load on the plot and that (2) the plot should be shielded by placing a net or some material partly filtering the light to ensure that the soil temperatures between both plots are equivalent.

ACKNOWLEDGEMENTS

This research was funded by Georgia Peanut Commission. We would like to thank the Royal Golden Jubilee (RGJ) Ph.D. program of Thailand Research Fund (TRF) and Chiang Mai University for providing a research scholarship. We especially appreciate the multi-faceted efforts of the staff at the Southwest Georgia Research and Education Center of the University of Georgia Plains Research Center for their logistical support of the present research. Authors are grateful to Professor Dr. John P. Beasley Jr, and Dr. Gengsheng Zhang for their kindly help and many recommendations in conducting this research.
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