● วารสารทางวิชาการของมหาวิทยาลัยเกษตรศาสตร์

วิทยาสารเกษตรศาสตร์

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Competition and Control of Weeds in Kale Leaf Crop

Tosapon Pornprom[1], Matthew Hayes[2] and Pramote Saridnirun[3]

ABSTRACT

Weed population responsed to weed control practices was carried out to evaluate weed seed bank, weed populations, and the effects of the herbicides applied preemergence of cultivation on kale leaf control. The first experiment involved an evaluation of the site including soil type and previous types of management. The seed bank was then evaluated to estimate both the composition and populations of weeds present, which could, then, be compared to the weeds growing. There was a positive correlation between the weed seed bank and above ground weed communities. The second experiment involved testing the effects of the herbicides at their recommended rates on weed control and the phytotoxicity on a kale leaf. The crop was most significantly affected by atrazine followed by oxyfluorfen and then alachlor. Three main weed species dominated the weed growth in this experiment were smooth mimosa (*Mimosa invisa*), slender amaranth (*Amaranthus viridis*) and purple nutsedge (*Cyperus rotundus*). The third experiment involved a bioassay testing, again of phytotoxicity of the three herbicides on nine separate crops; sweet corn, soybean, mungbean, swamp morning glory, cucumber, tomato, pepper, cabbage and coriander. Cabbage and coriander were not affected by any herbicides.

Key words: kale leaf (*Brassica oleracea* L.), alachlor, atrazine, oxyfluorfen, weed seed bank, weed control, weed population

INTRODUCTION

Species composition and population of weed communities varied greatly and are closely linked to control practices and cropping history. Composition is influenced by farming practices and varies from field to field and among areas within field (Buhler, 1999). The largest source of seeds which comprises the seed bank comes from plants that escape control and produce seeds within the field. Determining the type and quantity of seeds in the soil is important to forecast weed population dynamics (Buhler *et al.*, 1997). Within the seed bank there is a wide range of weed species present. However, in most cases a few dominant weed species will produce most of the seeds. Weed species become dominant when they are relatively resistant to weed control and well adapted to the conditions within the crop. Controlling these species should be the primary focus of weed control.

Kale leaf (*Brassica oleracea* L.) has been established throughout Asian countries as a significant crop in the past two centuries, being

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brought in from Europe. Kale leaf can be transplanted, but most of the production is direct-seeded into heavy, friable loam soils, with preferable pH of 6.5 to 6.8, as they produce the highest yields (Hodges, 1995). Commercially, the crop is produced for a one-time harvest around 40-55 days after planting with sequential plantings at 2-week intervals, to provide continuous supply for the market (Hall, 1995). In kale leaf crops in Thailand, much of the weed control still revolves around hand weeding. However, as this is becoming a less economically viable option more herbicides are being used. Three more commonly used herbicides are alachlor, atrazine and oxyfluorfen.

Alachlor can be applied mainly as a selective preemergence (PRE) herbicide to control a wide range of annual grasses, broadleaf weeds, and yellow nutsedge in corn and soybean (Thomson, 1993). The site of action is not known however a currently viable hypothesis involves the conjugation of acetyl coenzyme A and other sulfhydryl-containing biomolecules (Ahrens, 1994). The length of weed control varies from 6 to 10 weeks depending on soil types and weather conditions, with weeds affected before emergence, but seed germination is not inhibited.

Atrazine interferes with photosynthesis in many annual broadleaf weeds and grasses, while corn, sorghum, sugarcane, and a few other crops are tolerant of the chemical's effects at the recommended concentrations (Thomson, 1993). Atrazine kills plants by binding to the Q_B-binding niche on the D1 protein of the photosystem II complex in chloroplast thylakoid membranes, thus blocking electron transport from Q_A to Q_B. This stops production of ATP and NADPH (all needed for plant growth) (Ahrens, 1994). Death occurs when the plant is starved because photosynthesis is stopped from bleaching of the plant's chlorophyll, or from the release of the radicals, highly reactive molecules. After application, atrazine continues to control sprouting weeds for 5-6 weeks, allowing the desired crop to become well established without weed competition for moisture, nutrients and sunlight.

Oxyfluorfen is registered for preemergence (PRE) or early postemergence (POST) application in broccoli crops to control grass and broadleaf weeds (Thomson, 1993). The target site for oxyfluorfen appears to be protoporphyrinogen oxidase (Protox), an enzyme of chlorophyll and heme biosynthesis catalyzing the oxidation of protoporphyrinogen IX to protoporphyrin IX, resulting in loss of chlorophyll and carotenoids and leaky membranes, causing the cells to dry out (Ahrens, 1994).

Registered or unregistered herbicides occasionally damage crops, delay and decrease growth, and reduce harvest yield and quality. There has been much qualitative and quantitative information published on herbicide damage to crops (Boethel et al., 1999; Donal, 1998). The objective of this research was to evaluate the weed seed bank and control of weeds in kale leaf crop. These analyses included descriptions of herbicide damage symptoms, visually rate crop damage and changes in plant height and biomass. If potential yield losses could be estimated early in the growing season, this information might help farmers improve crop management decisions.

MATERIALS AND METHODS

Evaluation of existing seed bank

The site, at the Field Laboratory of the Department of Horticulture, Kasetsart University, Kamphaeng Sean Campus, used for growing the kale leaf had previously only been used for pasture and no herbicides had been used. Weed control methods used in the past were hand weeding and conventional tillage practices. A soil analysis was conducted in the plot area with four random samples taken, showing the soil to be a clay loam, pH 6.85; E.C. of 4.66 mS/cm; and an organic matter content of 2.9%. The plot had a net covering creating a greenhouse environment and more significantly protecting the plant from insect damage.

Temperatures were above 30°C in the greenhouse during the days and fell to around 25°C at night. Relative humidity was always above 85%.

To gain an accurate indication of the weed seed bank in the area, random soil samples were taken in a 50 m by 50 m site directly adjacent to the plot. In this site, 10 random 5 m by 5 m squares were allocated, within these squares, 5 soil samples were taken down to a depth of 15 cm. These samples were mixed together into 1 sample, and from this 1 sample, three equal amounts of soil were taken. This created three replicates at each of the ten squares. To gain an accurate indication of the seed bank within the plot area of 10 m by 20 m, the area was divided into four parts, with one random sample taken from the each part. These four samples were mixed to create 1 sample and from this 1 sample, three replicates were taken. Each soil replicate from the plot and adjacent area was placed in trays and placed in a greenhouse, which provided protection and the weeds were therefore under the same conditions as those in experiment 2. The trays were 0.125 m^2 by 5 cm deep. The replicates were watered regularly and this allowed the seeds within the soil to germinate and grow. From the plants that grew in the trays, the weed species composition and population were analysed. The trays were analysed three times, with the final survey 6 weeks after the initial soil collection. A weed survey was completed in places where soil samples were taken from, only the species composition was taken, not density.

Response of kale leaf and weeds to herbicides

The kale leaf cultivar Bang Buatong, was direct-seeded into the plot at a rate of 1 g/m^2. Plot preparation involved hand cultivation just prior to sowing. The plot was laid out into 15 separate subplots with 5 different treatments, three replications of each treatment. Within the layout each treatment was randomly allocated within a replication row, creating three replications containing each of the five treatments (randomised block design). The different treatments were a weedy control, hand weeding treatment and three different herbicides: alachlor at 3.75 kg ai/ha, atrazine at 3.13 kg ai/ha, and oxyfluorfen at 1.56 kg ai/ha. Each herbicide, a commercial formulation, was sprayed onto the subplots directly after sowing by a knapsack spray volume of 500 L/ha. These herbicide application rates were as recommended by the manufacturer. The hand weeding treatment involved weeding 7 and 14 days after sowing (DAS). The plots were watered twice daily, partly due to the dry conditions and the extremely hot and humid conditions in the greenhouse. At intervals of 14 and 21 DAS weed species population and composition was analysed on each subplot. A 50 cm square was placed randomly in each subplot and the numbers of weeds per square metre. 28 DAS a visual rating for weed control was given for all treatments, 0 = no weed control and 10 = complete weed control. Crop phytotoxicity to each treatment was treated 14 and 21 DAS, this involved weighing and the height of a sample of five plants. Only the shoots of the plants were measured for weight with the roots cut off.

Phytotoxicity of herbicides on crops

Nine representative crops were grown in soil from the three herbicide treatments to test phytotoxicity. The crops used were sweet corn (var. ATS-2), soybean (var. SJ 4), mungbean (var. KPS 1), swamp morning glory (var. Tupai 7), cucumber (var. Plong), tomato (var. Sridatip 3), pepper (var. Bangchang), cabbage (var. Speed 047), and coriander (var. Carlbe). These crops were direct-seeded into soil treated with herbicides (alachlor, atrazine and oxyfluorfen) and a control which was a combination of the hand weeding and weedy control (untreated) treatments. Treatments were replicated three times. The soil was taken from the relevant subplots down to a depth of around 2.5 cm within the plot and placed into 200 ml polyethylene pots in a shaded area. Each replicate of the experiment contained 25 seeds of the crop, which was to be sown in that row. This experiment was

repeated 0, 7, 14 and 21 DAS to give an estimate of how long the different herbicides would affect crop plants. To analyse the effect on the crop plants, the number of plants germinated was counted along with the height of five plants within each replicate to give a sample. A scoring system was used to assess crop injury caused by the herbicides. Crop injury was on a scale of 0 to 100; where 0 = no injury, 30 = moderate cotyledon necrosis and chlorosis and growth reduction, 70 = severe cotyledon injury and growth reduction, and 100 = all seedlings dead. The crop plants were analysed 10 DAS to allow a reasonable level of growth.

RESULTS

Evaluation of existing seed bank

Above ground, surveys were taken both in the field and within the plot. When comparing the above ground survey with the seed bank for the field sampled, four extra species were found in the seed bank, two broadleaf weeds and two grasses. The aboveground survey and seed bank composition of the plot were very similar.

In comparing the species composition of the field seed bank and the seed bank within the plot, more different species growing from seeds were collected from the field. Twelve extra broadleaf species and four extra grasses were present in the field seed bank compared with the plot seed bank.

When comparing the different populations of the weeds within the seed bank of the greenhouse and the field some differences became apparent (Table 1). In the greenhouse and field, the dominant weed population tended to differ. In the field seed bank, the broadleaf species *Portulaca oleracea* L., and *Eclipta alba* L. were dominant, whilst in the greenhouse *Amaranthus viridis* L., and *Euphorbia thymifolia* L. were at the hightest levels amongst the broadleaf weeds. Of the grasses, *Echinochloa crusgalli* L. was the dominant weed in the field, whilst in the greenhouse *Dactyloctenium aegyptium* was the major grass weed. The one sedge species *Cyperus*

rotundus L. was present at much higher levels in the field seed bank. It was reasonable to suggest that there was a positive correlation between the weed seed bank and aboveground weed communities.

Response of kale leaf and weeds to herbicides

The results showed that the three herbicides caused a significant fall in kale leaf weight and plant height in accordance to the weedy control. Similar trends were observed (Figure 1), so the plant height data were omitted. The hand weeding and weedy control treatment had similar plant height and weight averages. Atrazine had the most detrimental effect on plant height and weight. Alachlor and oxyfluorfen affected the weight and height of the kale leaf plants at similar amounts but significantly less than the atrazine treatment.

In the plot the weeds were mainly broadleaf species and one sedge species with only relatively small numbers of grasses. Three species, two broadleaf species and one sedge, dominated the weed counts and were in even higher numbers in the control. The species were amaranth, mimosa and sedge, with amaranth clearly the highest in number in the weedy control (Figure 2). Many other species were found in the control but only in very small numbers from the herbicide treatments. The three main species were found in highest numbers in the control as expected. The alachlor and oxyfluorfen treatment had similar levels of control for mimosa and sedge. The oxyfluorfen treatment had a much greater control for amaranth compared with in the alachlor treatment. The atrazine treatment had a much greater control on sedge and mimosa compared with the other treatments and had a similar levels of control on amaranth when compared with the oxyfluorfen treatment.

Phytotoxicity of herbicides on crops

Four sets of results for germination and plant height were taken where the crops were sown 0, 7, 14, and 21 DAS. The results 14 and 21 DAS were not used in the analysis as there were no

Table 1 Above ground weed species identified in the field and weed seed bank in the greenhouse.

Weed species	Density (per m²)		Weed species	Density (per m²)	
	Above ground	Seed bank		Above ground	Seed bank
Broadleaf weeds :			**Grass weeds :**		
1. *Abutilon hirtum* Sweeet.	0.3	-	21. *Brachiarai mutica* Forssk. Stapf.	5.3	2.1
2. *Aeschynomene americaca* L.	0.3	-	22. *Brachiarai reptans* L. Grad. Et Hubb.	0.3	-
3. *Ageraturm conyzoides* L.	0.3	-	23. *Cynodon dactylon* L. Pers.	0.5	-
4. *Amaranthus spinosus* L.	0.5	-	24. *Dactyloctenium aegyptium* L. P.B.	2.9	12.0
5. *Amaranthus viridis* L.	1.9	40.0	25. *Digitaria ciliaris* Retz. Koel.	0.5	-
6. *Boerhavia diffusa* L.	0.8	2.7	26. *Echinochloa colona* L. Link.	13.0	2.1
7. *Cleome viscosa* L.	2.7	2.7	27. *Echinochloa crus-galli* L. Beauv.	43.0	1.9
8. *Corchorus aestuans* L.	0.8	-	28. *Eleusine indica* L. Gaertn.	5.9	-
9. *Eclipta aiba* L. Hassk.	42.0	2.7	29. *Ischaemum rugosum* Salisb.	1.1	1.3
10. *Euphorbia thymifolia* L.	7.2	21.0	30. *Leptochloa chinensis* L. Nees.	0.5	1.3
11. *Heliotropium indicum* L.	0.3	-	31. *Panicum repens* L.	2.9	1.9
12. *Ipomoea aquatica* Forssk.	0.3	-			
13. *Ipomoea pestigridis* L.	1.0	-	**Sedge :**		
14. *Macroptilium lathyroides* L. Urb.	0.3	-	32. *Cyperus rotundus* L.	15.0	1.3
15. *Mimosa invisa* Mart.	1.1	-			
16. *Mimosa invisa* Var. inermis Adelb.	0.5	8.0			
17. *Phyllathus ninuri* Auct.	0.5	-			
18. *Portulaca oleracea* L.	19.0	2.7			
19. *Pysalis minima* L.	1.6	2.7			
20. *Trianthema porutlacastrum* L.	0.3	2.7			

significant herbicide effects this long after application. To establish if there were any significant effects by herbicide, a one-way ANOVA was completed.

0 DAS the germination percentages of cucumber and tomato were significantly lowered by atrazine. Alachlor and oxyfluorfen did not significantly lower the germination percentages of the nine crops 0 DAS (Figure 3). 7 DAS sweet corn germination percentage was significantly lowered by alachlor and oxyfluorfen (Figure 4). Soybean germination was significantly lowered by atrazine, whilst pepper germination was significantly lowered by both atrazine and oxyfluorfen. When compared with the germination, 0 DAS the cucumber and tomato affected by atrazine were not significantly affected 7 DAS. Not until 7 DAS did all three herbicides began to affect the germination of the crops. Atrazine had the greatest effect on germination percentage followed by oxyfluorfen and then alachlor.

From the results, it could be seen that all herbicides had a more significant effect on plant height than germination. 0 DAS, cucumber was significantly affected by all three herbicides;

soybean, mungbean and cucumber significantly affected by the atrazine; and sweet corn, mungbean, and cucumber by oxyfluorfen (Figure 5). 7 DAS the height of mungbean and swamp morning glory were significantly affected by all three herbicides, and the plant height of cucumber was significantly affected by atrazine (Figure 6). The plant height of 5 out of the nine crops were affected by herbicides: sweet corn, soybean, mungbean, swamp morning glory and cucumber. Of the three herbicides, atrazine lowered the mean heights of the plants to the greatest extent, followed by oxyfluorfen and then alachlor.

DISCUSSION

Determining the type and quantity of weed seed bank is important in forecasting weed population dynamics (James and Rahman, 1999). The result showing more different species present in the field seed bank than in the greenhouse were expected as the soil samples from the field covering a much greater area. As only 2 to 6% of weed seeds produced ever develop into seedling (Ball and Miller, 1989), some variation in field surveys and

Figure 1 Growth response of kale leaf to different weed control treatments. Alachlor was applied at 3.75 kg ai/ha, atrazine at 3.13 kg ai/ha, and oxyfluorfen at 1.56 kg ai/ha. Vertical bars indicate the standard error of the mean.
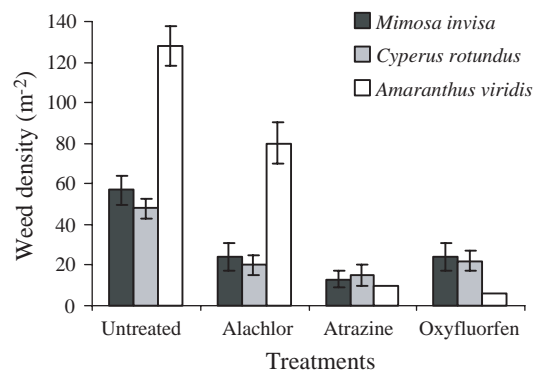
Figure 2 Density of three main weed species in separate treatments. Alachlor was applied at 3.75 kg ai/ha, atrazine at 3.13 kg ai/ha, and oxyfluorfen at 1.56 kg ai/ha. Vertical bars indicate the standard error of the mean.
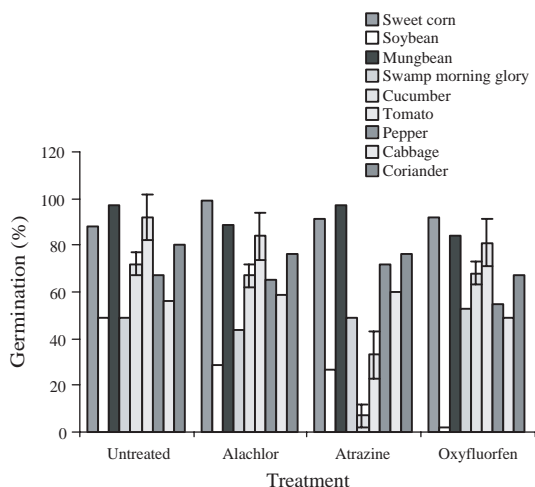
Figure 3 Germination percentages of the nine crops sown on day of herbicide application. Alachlor was applied at 3.75 kg ai/ha, atrazine at 3.13 kg ai/ha, and oxyfluorfen at 1.56 kg ai/ha. Vertical bars indicate the standard error of the mean.
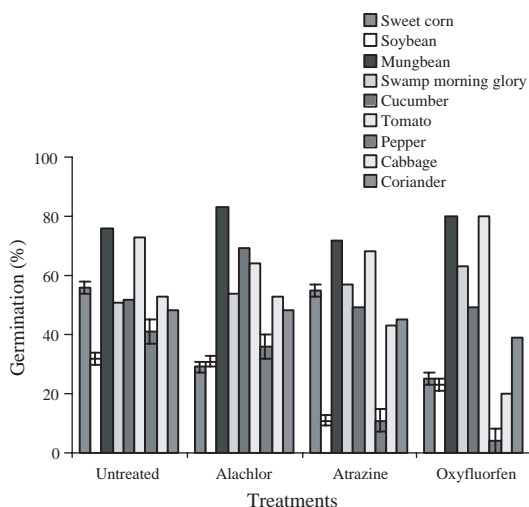
Figure 4 Germination percentages of the nine crops sown at 7 days after sowing. Alachlor was applied at 3.75 kg ai/ha, atrazine at 3.13 kg ai/ha, and oxyfluorfen at 1.56 kg ai/ha. Vertical bars indicate the standard error of the mean.

Figure 5 Plant height (cm) of the nine crops sown on day of herbicide application. Alachlor was applied at 3.75 kg ai/ha, atrazine at 3.13 kg ai/ha, and oxyfluorfen at 1.56 kg ai/ha. Vertical bars indicate the standard error of the mean.

Figure 6 Plant height (cm) of the nine crops sown at 7 days after sowing. Alachlor was applied at 3.75 kg ai/ha, atrazine at 3.13 kg ai/ha, and oxyfluorfen at 1.56 kg ai/ha. Vertical bars indicate the standard error of the mean.
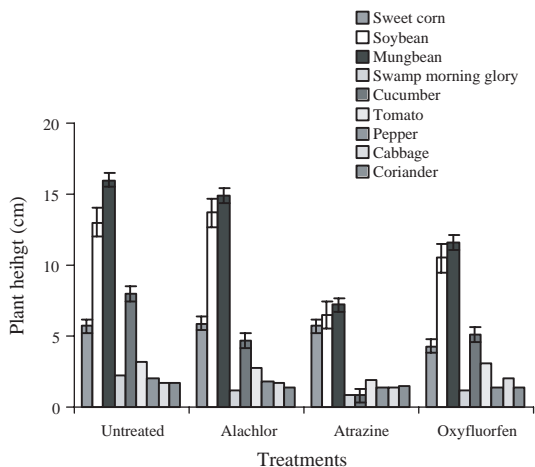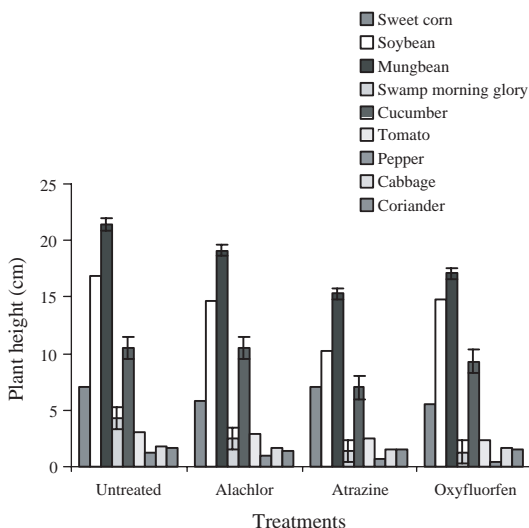
seed bank composition would be expected. All of the weed species present in the plot seed bank were found in the field seed bank showing that the plot gave a good indication of the weeds present in the area. As expected, more weed species were present in the field seed bank as this survey covered a much larger area and this area underwent differing management practices. Certain broadleaf and grass species dominated the weed population both in the field and the plot seed bank. Although seed banks and the resulting weed populations are composed of many species, a few dominant species generally comprise 70 to 90% of the total seed bank. In general, short-lived plants such as annual broadleaf and grass species have long-lived seeds and substantial seed banks. Seed can remain dormant in the soil for many years (e.g., several years to decades, or longer, depending on the plant species and environmental conditions), with only a fraction of the seeds germinating each year.

When comparing the weed populations of the plot seed bank and the field real patterns emerged. Some broadleaf species were at higher levels in the field and others in the greenhouse, the same was found with the grasses. The one sedge species was found in much higher levels in the field. This might be due to spraying in the field which controlled other species but not sedge. Spraying has a very limited control on sedge species (Patterson, 1998). The differing compositions of the greenhouse and the field could also be due to control methods such as spraying used in the field, whilst no spraying took place greenhouse.

The hand weeding treatment and weedy control had similar height and weight measurements for the kale leaf plants. It would be expected that kale leaf in the hand weeding treatment would be heavier and taller as they only had to compete with low levels of weeds. In the weedy control, there were higher levels of weeds but competition was not a problem as there was high levels of water and nutrients accounting for the similarities in the treatments. Atrazine had the most severe effect on

growth of the plants. As these measurements were taken 28 DAS and the herbicides were sprayed PRE, these herbicide effects would be found right through to harvest. This effect of atrazine would suggest that it was not suitable to use in the kale leaf. Although the alachlor and oxyfluorfen do affect the kale leaf growth, these may be less phytotoxic at lower concentrations. In another experiment on oxyfluorfen effect on *Brassica oleracea* L., it was found that injury ranged from cotyledon crinkling and slight growth reduction at the lower rates to severe growth reduction, cotyledon necrosis, and seedling death at the higher rates (Harrison and Farnham, 1998). This suggests that alachlor may be the most suitable to use in kale leaf purely on the basis that it is the least phytotoxic to the crop.

The weed species composition found in the weedy control treatment of experiment 2 was comparable to the species found in the seed bank analysis, with more species found in the seed bank analysis. Of the weeds growing in the plot, the three dominated species were mimosa (*Mimosa invisa* Var. inermis Adelb.), sedge (*Cyperus rotundus* L.) and amaranth (*Amaranthus viridis* L.). It would not be expected that these three species were in high numbers because of resistance to the herbicides as the greenhouse had not been sprayed previously. Of these three species, amaranth was the only species which was at high level within the seed bank, suggesting that the other two species were highly competitive in this environment. One other broadleaf, *Euphorbia thymifolia* L., and one grass species *Dactyloctenium aegyptium* were present at high levels in the seed bank analysis but not in the weedy control in the plot showing the variability in weed growth from the seed bank.

Of the nine crop analysis, neither germination percentage nor plant height of cabbage and coriander were affected by any of the three herbicides. This meant that the three herbicides could be used in these crops, with atrazine the ideal choice as it had the greatest weed control. Atrazine affected either the plant height or germination of all the remaining

crops asides from sweet corn, rendering it unusable in these crops. In other studies, it has been found that the corn is tolerant to the chemicals effects at recommended applications. Corn, a crop on which atrazine is heavily used, is resistant to atrazine because it can detoxify the posion by means of an enzyme present in its leaves. In addition, corn roots contain a substance that helps break down atrazine molecules. It has also been found that sensitive crops such as soybean can be affected in the following growing season when atrazine has been sprayed the previous year.

Of the three herbicide, alachlor caused the least phytotoxic. The germination and plant height of tomato, pepper and soybean were not affected by alachlor, suggesting that this herbicide would be suitable to use in these crops. This herbicide will generally persist long enough in the soil to provide 6-10 weeks of weed control depending on soil type and weather conditions (Ahrens, 1994). It has moderate mobility in sandy soil and thus can migrate to groundwater. Oxyfluorfen did not affect the germination or plant height of soybean and tomato making it a suitable herbicide for these crops. This herbicide provides weed control for around two months. It is immobile on most soils, but slightly mobile on extremely sandy soils. Oxyfluorfen has a strong tendency to adsorb soil particles and is nearly insoluble in water. Once oxyfluorfen is adsorbed to soil particles, it is not readily removed. It is, therefore, unlikely to leach downward or to contaminate groundwater.

Although herbicide use has been shown to be successful, it should coincide with the presence of sufficient weeds to warrant use and take place when weeds are most vulnerable. Factors which must be considered when developing a herbicide program are the herbicide itself, weed flora and application time, crop tolerance and cost effectiveness. Furthermore, adjusting sowing dates of crops could avoid weed emergence peaks and thus minimise yield losses from weed competition (Clay *et al.*, 1999; Del Monte *et al.*, 1999). It is

suggested that estimates of seed bank populations in arable soils could also be used to predict future weed infestations (Callihan and Dobbins, 1996). Such information would have value in planning crop sequences and herbicide usage.

Crop production systems are dependent on management options which successfully reduce the effect that weeds have on crops. Herbicides reduce weed density and indirectly reduce weed seeds that are produced and enter the seed bank. Although herbicides are effective in controlling weeds, an increasing environmental awareness has created a desire to reduce the amount of herbicides applied to agricultural fields. Integrated weed management practices used in Thailand include good land preparation, suitable rates and timing of planting, well-times hand weeding, effective water management, chemical herbicides when necessary and more recently crop rotation.

CONCLUSION

In the seed bank analysis and above ground surveys the species compositions were very similar as expected. The seed bank analysis of the greenhouse differed from the field seed bank both in weed species composition and population. This was as expected since the field seed bank covered a much wider area and the conditions in the greenhouse, were much different to the field and favoured the broadleaf species which tended to dominate. Of the weed counts taken out within the greenhouse, three dominant species were two broadleaf, amaranth and mimosa and one sedge species. Atrazine provided the greatest control of these species followed by oxyfluorfen and alachlor. However, atrazine was the most detrimental of the three herbicides to the growth of the kale leaf followed by oxyfluorfen and alachlor. Among nine crops under study, atrazine had the most detrimental effect on germination and plant height followed by oxyfluorfen and alachlor. Cabbage and coriander were not affected by the herbicides.

LITERATURE CITED

Ahrens, W.H. 1994. Herbicide Handbook, 7th ed. Illinois, 352 p.

Ball, D.A. and S.D. Miller. 1989. A comparison of techniques for estimation of arable soil seed banks and their relationship to weed flora. Weed Res. 29 : 365-373.

Boethel, D.J., J.L. Griffin, C.F. Grymes, D.L. Jordan, B.R. Leonard, and J.S. Russin. 1999. Soybean response to weed interference and defoliation. Weed Sci. 47 : 90-94.

Buhler, D.D. 1999. Weed population responses to weed control practices. I. Seed bank, weed populations, and crop yields. Weed Sci. 47 : 416-422.

Buhler, D.D., R.G. Harzler, and F. Forcella. 1997. Implications of weed seed bank dynamics to weed management. Weed Sci. 45 : 329-336.

Callihan, R.H. and R.T. Dobbins. 1996. Weeds diagnostic laboratory. Department of PSES, University of Idaho. Available Source : http://www.catalinaconservancy.org/er/weeds/weedplan.htm, 15 October, 2000.

Clay, S., F. Forcella, and G.O. Kegode. 1999. Influence of crop rotation, tillage, and management inputs on weed seed production. Weed Sci. 47 : 175-183.

Del Monte, J.P., J. Dorado, and C. Lopez-Fando. 1999. Weed seed bank response to crop rotation and tillage in agroecosystems. Weed Sci. 47 : 67-73.

Donald, D.D. 1998. Estimated soybean (*Glycine max*) yield loss from herbicide damage using ground cover or rate stunting. Weed Sci. 46 : 454-458.

Hall, M. 1995. Brassica crops. Available Source : http://www.cas.psu.edu/docs/casdept/agronomy/forage/dosc/species/brassica.htm, 1 November, 2000.

Harrison, F.H. Jr. and M.W. Farnham. 1998. Differential tolerance of broccoli (*Brassica oleracea*) cultivars to preemergence application of oxyfluorfen. Weed Technology. 12 : 14-18.

Hodges, L. 1995. Kale : The "New" Old Vegetable. University of Nebraska. Available Source : http://www.iarn.unl.edu/pubs/hoticulture/nf53.htm, 30 October, 2000.

James, T.K. and A. Rahman. 1999. Evaluation of 2 methods for Enumerating. The Soil Weed Seed Bank. Available Source : http://www.hrtnet.co.nz/publications/nzpps/proceedings/95/95_175.htm, 10 November, 2000.

Patterson, D.T. 1998. Suppression of purple nutsedge (*Cyperus rotundus*) with polyethylene film mulch. Weed Technology 12 : 275-280.

Thomson, W.T. 1993. Agricultural Chemicals, Book II Herbicides. Thomson publications, Fresno. 309 p.

Spatial Distribution Pattern of Cotton Leafhopper, *Amrasca biguttula* (Ishida) (Homoptera: Cicadellidae)

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ABSTRACT

The spatial distribution pattern of cotton leafhopper, *Amrasca biguttula* (Ishida) was studied under field conditions during 2000 and 2001 at Suwan Farm, Pak Chong, Nakon Ratchasima, Northeastern Thailand. The cotton varieties/lines: Sri Samrong 60 (the recommended variety), Sarid1 and new mutant lines AP1 and AP2 were used in the experiments employing Randomized Complete Block design with 4 replications. The results of both seasons showed that the distribution of *A. biguttula* was clumped for all varieties/lines and degree of aggregation considerably changed during the generations, as indicated by the values of variance to mean ratio ($s^2/\overline{x}$), negative binomial parameter (k) and the index of aggregation ($I\delta$). The results of distribution analysis were used to estimate a given sample size and precision level of cotton leafhopper. The appropriate sample size of maximum 10 and 30 plants could be required at low and high aggregation levels of *A. biguttula*, respectively.

Key words: cotton leafhopper , *Amrasca biguttula* , distribution

INTRODUCTION

Information on distribution pattern of insect pests was used in data analysis to determine appropriate sampling plan and sample size, and constructed sequential sampling programs. Insect populations were mostly aggregated; some were found to be either random or uniform dispersions (Southwood, 1978; Mabbett, 1980; Taylor 1984; Davis, 1994). It was found that cotton arthropods generally exhibited a clumped pattern of dispersion, which was often characterized by fit to the negative binomial distribution (Wilson *et al.*, 1989). Several methods have been used to describe the distribution of insect counts. Kapatos *et al.* (1997) reported that the Variance mean ratio ($s^2/\overline{x}$) and the Morisita's

Index ($I\delta$) were found best to describe for classifying spatial distribution pattern during the generations compared with the Taylor relationship. The negative binomial parameter k was described as a degree of clumping and low values of k indicated a higher degree of clumping (Southwood, 1978; Davis, 1994).

The cotton leafhopper, *Amrasca biguttula* (Ishida) (Homoptera: Cicadellidae) is one of the most alarming key pests of cotton in Thailand. The nymphs and adults suck the sap from leaves and cause phytotoxic symptoms known as hopperburn which results in complete desiccation of plants. *A. biguttula* is an early phase pest of cotton. Recently, it occurs throughout the cotton growing season and has become one of the limiting factors in economic

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productivity of the crop. As most cotton growers depend on chemical insecticides for controlling leafhopper, the necessity for more accurate population assessments of this pest become more acute.

Previous studies mentioned that the distribution of *A. biguttula* showed aggregation and could be adequately described by the negative binomial distribution (Mabbett, 1979; Mabbett and Nachapong, 1980; Wilson and Room, 1983; Mabbett *et al*., 1984; Matthews 1989, 1994). These findings agreed by Evans (1965) who investigated the distribution of leafhopper in Sudan. Mabbett *et al*. (1984) suggested that sequential sampling could be used based on binomial sampling theory. Sample units would be restricted to the infestation sites and treatment decision would be made on the proportion of sample units rather than on actual pest counts. The cost of monitoring can also be reduced by sampling units where the pest is most frequently found (Wilson, *et al*., 1982).

Many variables, such as soil moisture, fertility, natural enemies, physiological time, weather, and others, can affect the numbers of pests and their distribution patterns (Pedigo *et al*., 1986 ; Davis, 1994). Therefore, it is still needed to evaluate the distribution pattern of cotton leafhopper for specific area, different seasons and different cotton varieties to make a correct decision of cotton pest management. The present study was designated to determine the distribution pattern of cotton leafhopper nymphs in the field leading to the development of optimum sampling plan.

MATERIALS AND METHODS

Field experiments were conducted at Suwan Farm, Pak Chong, Nakon Ratchasima Province for two growing seasons, the first crop (1 October 2000 to 15 March 2001) and the second crop (21 July 2001 to 27 Dec 2001). The soil texture was clay and pH was 7-7.5. In both years, cotton varieties: Sri Samrong 60 (SR60), Sarid1 (SD1) and new mutant lines AP1 and AP2 obtained from gamma-irradiated SD1 were planted as treatments. Agricultural practices adopted in this experiment were treated as required. Each variety/line was grown in 7 rows, each row of 20-meter long. Spacing of row × plant was 1.0×1.0 meters. Plots and blocks were separated by 2 m each of unplanted areas. The total experimental area was 2,924 m^2. Randomized Complete Block (RCB) design was employed with 4 replications. At planting time, 38 kg/ha Furadan 3% G and 0.06 kg/ha Imidacloprid 70% WS were applied in the first and second crops, respectively. 50 ml per 20 litres (l) of water Carbosulfan 20% EC and 40 ml/20 l of water Omethoate 50% SL were applied 5,6,7,8 weeks after sowing(WAS), alternately for first crop and 40 ml per 20 l of water Azodrin 60% WSC applied once 4 WAS for second crop, to control severe attack of leafhopper.

Observations were made 5 WAS and continued at weekly intervals till cotton was 3 months old. Visual counts of leafhopper nymphs were only emphasised on 5 leaves/plant (one from the top, two each from the middle and bottom of the canopy)(Evans, 1965; Mabbett, 1980). Stratified random sampling technique was designated on 10 plants to give a total of 160 sample plants. Mean number of leafhopper per leaf was then calculated. Outer rows from every plot were not selected for sampling. Inspections of leafhoppers per plant were fitted to distributions which would be expected if leafhopper nymphs randomly spreaded (poisson distribution) or aggregated (negative binomial distribution).

The distribution pattern of cotton leafhopper was statistically classified by calculating the Variance mean ratio ($s^2 / \overline{x}$) and Morisita's Index ($I\delta$), the mean ($\overline{x}$), sample variance (s^2) and size of the sampling unit (n) (Morisita, 1962; Southwood, 1978; Davis, 1994). The negative binomial parameter *k* (a measure of dispersion) was calculated as degree of clumping for each sampling date after classifying the data into frequency distribution. It is inversely related to the degree of aggregation,

whereas the opposite holds for $I\delta$ (Southwood, 1978).

The number of samples (n) necessary to estimate the mean with fixed precision was determined as suggested by Southwood (1978). When the confidence limits were used as the predetermined standard, the required half-width was usually set at 10%, then adjusted sample unit (n') was calculated. The calculations were based on the 4 varieties/lines altogether with 7 sampling dates (5 WAS – 11 WAS).

In the first crop, the total rainfall for the planting period (October 2000 to March 2001) was recorded as 397.3 mm and no rain was recorded in December 2000. In the second crop, the total rainfall for planting period (July 2001 to December 2001) was recorded as 489.7 mm and no rain was recorded

in December. In the first crop, the minimum and maximum temperatures were 21.08°C and 30.28°C, respectively while in the second crop, they were 21.73°C and 31°C, respectively.

RESULTS AND DISCUSSIONS

Spatial distribution pattern of cotton leafhopper was investigated in the field to provide the informations necessary for development of appropriate sampling plan and optimum sample size for cotton pest management. Table 1 and 2 present the distribution indices ; Variance mean ratio, Morisita's Index and the negative binomial parameter k of each variety/line of the two growing seasons. It was found that both $[(s^2/\overline{x})$ and $(I\delta)]$ showed significantly greater than 1.0 for all cotton

Table 1 Evaluation of spatial distribution pattern of *Amrasca biguttula* (Ishida) nymphs for four cotton varieties/lines at weekly intervals (5 WAS - 11 WAS) during the first crop at Suwan Farm.

Variety /line	Variance mean ratio $(S^2/\overline{x})$	t-value [1]	Distribution pattern	Morisita's Index (Id)	F_o [1]	k-value	Distribution pattern
AP1	8.05	13.58	clumped	1.73	8.05	1.35	clumped
AP2	5.32	10.28	"	1.46	5.32	2.15	"
SD1	8.19	13.57	"	1.73	8.19	1.36	"
SR60	6.79	11.13	"	1.52	6.79	1.90	"

[1] significant at p = 0.01
WAS = Weeks After Sowing

Table 2 Evaluation of spatial distribution pattern of *Amrasca biguttula* (Ishida) nymphs for four cotton varieties/lines at weekly intervals (5 WAS- 11 WAS) during the second crop at Suwan Farm.

Variety /line	Variance mean ratio $(S^2/\overline{x})$	t-value [1]	Distribution pattern	Morisita's Index (Id)	F_o [1]	k-value	Distribution pattern
AP1	8.24	22.63	clumped	5.21	8.24	0.24	clumped
AP2	14.74	20.82	"	4.34	14.74	0.29	"
SD1	10.00	20.45	"	4.32	10.00	0.30	"
SR60	16.23	19.05	"	3.75	16.23	0.36	"

[1] significant at p=0.01
WAS = Weeks After Sowing

varieties/lines during the investigation period indicating clumped distribution. Furtherly, the results were confirmed by t-value and F_o value (P = 0.01) (Table 1 and 2). The studies clearly showed clumped distribution pattern of leafhopper in the field. It could be assumed that cotton variety/line and growing season did not affect the distribution pattern of leafhopper. It also indicated that cotton arthropods generally exhibited a clumped pattern of dispersion, which was often characterized by fit to the negative binomial distribution (Wilson *et al.*, 1989). The similar findings agreed with Evans (1965) and Mabbett *et al.* (1984) who reported the distribution of leafhopper on cotton in Sudan and Thailand showing aggregation and could be adequately described by the negative binomial distribution.

The general evaluations of spatial distribution of leafhopper for both crops were described in Table 3. In both seasons, $s^2/\bar{x}$ and Id showed significantly greater than1.0 indicating clumped distribution. The results were also confirmed by t-value and F_o. Comparing the *k* values of each variety/line in both seasons, it was obvious that populations were highly aggregated (k=0.27) in the second crop with the *k* value of 1.62 in the first crop (Table 3). Higher temperature possibly caused the result in greater aggregation of insects in the second crop. It was also possible due to the fact that different management practices applied throughout the cropping season such as frequency of insecticide applications in the first

crop were much more than those of the second crop in controlling early infestation of leafhopper apart from soil fertility, application of nitrogen fertilizer and weather (Pedigo *et al.*, 1986; Davis, 1994).

The values of Iδ for the first crop were found to clearly indicate a considerably low in the amount of aggregation of the insects during early growing stages (5 -7 WAS) of cotton (Figure 1) owing to the applications of insecticide in such periods. It was noticed that the values fluctuated up to 11 WAS except AP1 whose peak (2.00) was at 10 WAS (Figure 1), whereas the reverse was observed for *k* (Figure 2). The high levels of *k* value of all varieties/lines were recorded at early growing stages (5 - 6 WAS) and decreased 7-8 WAS except SD1 (7 WAS), then fluctuating continued until 10 WAS. Quite a number of natural enemies was found which might result in low aggregation of the leafhopper. Thereafter, the *k* value of each one sharply increased again at late growing stage (11 WAS). The results showed 2 distinct peaks of aggregation, one 8 WAS(early December) and the other 10 WAS(late December) (Figure 2) which might be caused by the appearance of the leafhopper's next generation. The populations seemed to be most aggregated when population densities were very low. Therefore, it could be concluded that the degree of aggregation changed considerably during a generation.

The trends of Iδ value of each variety/line were observed to be inconsistently fluctuated in the second crop (Figure 3). The initial indices were low

Table 3 Overall spatial distribution pattern of *Amrasca biguttula* (Ishida) nymphs for four cotton varieties/lines in the first and the second crops at Suwan Farm.

Crop	Variance mean ratio($S^2/\bar{x}$)	t-value [1]	Distribution pattern	Morisita's Index (Iδ)	F_o [1]	k-value	Distribution pattern
First	7.12	24.48	clumped	1.61	7.12	1.62	clumped
Second	14.13	31.26	"	4.74	14.13	0.27	"

[1] significant at p=0.01

WAS = Weeks After Sowing

5 WAS and increased 6 WAS and then decreased and increased again. The highest index (10.38) was recorded in AP1 11 WAS. The indices of Iδ of the four varieties/lines in the second crop were higher than those in the first crop ca. three times. The reversed results were also observed for k values (Figure 4). The initial values were high 5 WAS and decreased 6 WAS and then sharply increased and decreased again. The populations were found to be highly aggregated 6 (early September), 9 (early October) and 11 WAS (late October), except SD1 (Figure 4).

Comparing those patterns, the difference was noticed between the two growing seasons. Southwood (1978) reported that k value could be influenced by predation, size of sampling units, and weather. According to Evans (1965), as the insects developed, nymphs migrated from plant to plant and the aggregation could be reduced. However, that differed from the results of the second crop since there was aggregation peak 11 WAS. It was recorded in Sudan that the distribution changed when the production of new leaves ceased resulting in an obvious decrease in the number of the first -

Figure 1 The Morisita's Indices (Iδ) of aggre-gation of *Amrasca biguttula* on four cot-ton varieties/lines at each sampling date in the first crop.

Figure 2 The negative binomial parameters (k) of four cotton varieties/lines at each sam-pling date in the first crop.
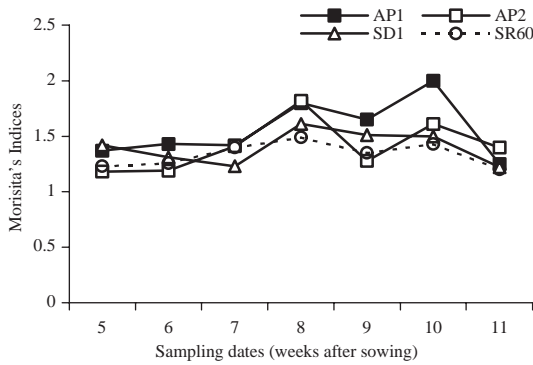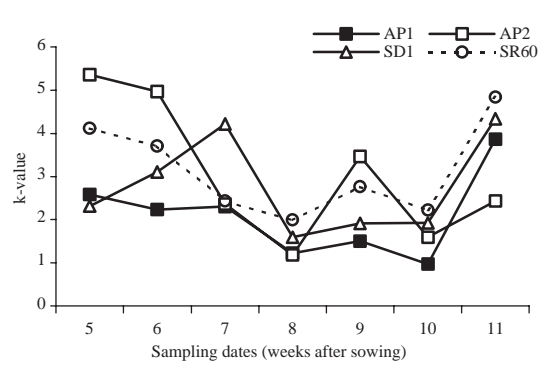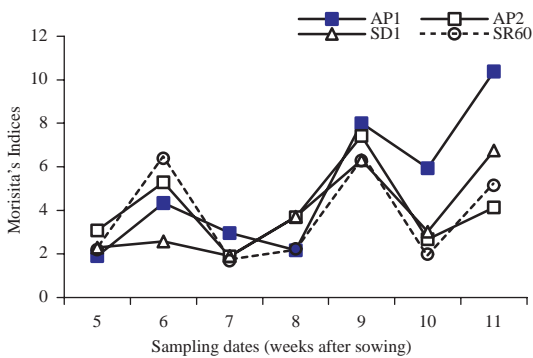

Figure 3 The Morisita's Indices (Iδ) of aggre-gation of *Amrasca biguttula* on four cot-ton varieties/ lines at each sampling date in the the second crop.
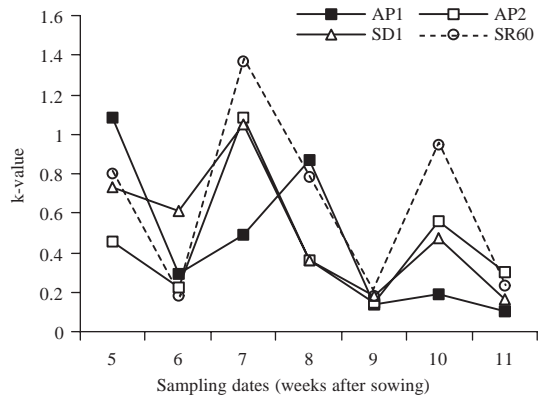
Figure 4 The negative binomial parameters (k) of four cotton varieties/lines at each sam-pling date in the second crop.

instar nymphs (Evans, 1963).

In accordance with the results, stratified random sampling technique and visual count sampling method could be appropriate for leafhopper scouting procedure. In addition, the sampling time and labor costs would be saved because only clumped distribution pattern was observed throughout the investigation period (5-11 WAS) of both crops. The optimum sample size, a maximum of 10 plants (p = 0.05) could induce low aggregation (k value higher than 1.0) of leafhopper. However, maximum of 30 plants (p = 0.05) should be monitored at high aggregation (k value lower than 1.0) level. The results confirm previous finding in showing that the clumped behavior of an insect affects the number of samples required to estimate the population density with a given level of reliability (Wilson and Room, 1983; Wilson, 1994). The population density can be monitored during the season and treatments can be delayed until the economic threshold is reached.

CONCLUSION

The distribution of cotton leafhopper expressed as clumped throughout the investigation period for both seasons. The changes of spatial pattern were described satisfactorily by the dispersion indices k and Iδ. The populations seemed most aggregated when population densities were very low. As the clumping behavior of *A. biguttula* affected the number of samples, therefore, it was reasonable to suggest that the optimum sample size of maximum 10 plants could be required at low aggregation and a considerably larger sample was required maximum 30 plants at high aggregation level of cotton leafhopper. It is possible that sampling frequency could be reduced to save time consuming and labor costs. Recommended sampling technique and appropriate sampling method are stratified random sampling and visual count of cotton leafhopper .

LITERATURE CITED

Davis, P. M. 1994. Statistics for describing populations, pp.33-54. *In* L. P. Pedigo and G. D. Buntin(eds.). Handbook of Sampling Methods for Arthropods in Agriculture. CRC Press. London.

Evans, D. E. 1963. Investigations on jassid biology. Prog. Rep. Exp. Stns Emp. Cott. Grow. Crop., Sudan , 19-24 pp.

Evans, D. E. 1965. The distribution of *Empoasca lybica* (Deberg) (Hemiptera: Cicadellidae) on cotton in the Sudan. Bull. Entomol. Research. 56 : 635-647.

Kapatos, E. T., E.T. Stratopoulou and A. Sahinoglou. 1997. Spatial pattern of *Saissetia oleae* (Homoptera: Coccidae) in Greece. Environ. Entomol. 26(6) : 1202-1207.

Mabbett, T.H. 1979. Guidelines for the management of cotton pests. Part 2. The cotton jassid (*Empoasca devastans* Distant). Dept. Agri., Technical Booklet No.2. Bangkok, 9 p.

Mabbett, T.H. 1980. Management of four major cotton pests. World crops. 32 (4) : 101-104.

Mabbett, T.H and M. Nachapong. 1980. The development of simple scouting techniques for the cotton jassid (*Amrasca devanstans* Distant). Thai J. Agric. Sci. 13 : 21-27.

Mabbett, T. H ., M. Nachapong, K. Monglakul, and J. Mekdang. 1984. Distribution on cotton of *Amrasca devastans* and *Ayyaria chaetophora* in relation to peat scouting techniques for Thailand. Tropical Pest Management. 30 (20) : 133-141.

Matthews, G. A. 1989. Cotton insect pests and

their management. Longman, London. 199 p.

_____ . 1994. Jassids (Hemiptera : Cicadellidae), pp. 353-357. *In* G. A. Matthew and J. P. Tunstall (eds.), Insect Pests of Cotton, CAB International, UK.

Morisita, M. 1962. Id index, a measure of dispersion of individuals, Res. Popul Ecol.4 : 1-7.

Pedigo L. P., S. C.Hutchins, and L. G. Higley. 1986. Economic enjury levels in theory and practice. Annu. Rev. Entomol. 2 : 341-368.

Southwood, T.R.E. 1978. Ecological Methods with Particular Reference to the Study of Insect Populations. ELBS and Chapman and Hall, London. 524 p.

Taylor, L. R. 1984. Assessing and interpreting the spatial distribution of insect populations. Annu. Rev. Entomol. 29 : 321-357.

Wilson, L. T. 1994. Estimating abundance, impact, and interactions among arthropods in cotton agroecosystem, pp.476-514. *In* L. P. Pedigo and G. D. Buntin(eds.). Handbook of Sampling Methods for Arthropods in Agriculture. CRC Press. London.

Wilson, L. T and P. M. Room. 1983. Clumping patterns of fruit and arthropods in cotton, with implication for binomial sampling. Environ. Entomol. 12(1) : 50-54.

Wilson, L. T., A. P. Gutierrez, and D. B. Hogg. 1982. Within-plant distribution of cabbage looper, *Tricoplusia ni* (Hubner) on cotton: development of a sampling plan for eggs. Environ. Entomol. 11 : 251-254.

Wilson, L. T., W.L., Sterling, D. R., Rummel, and J. E., DeVay. 1989. Quantitive sampling principles in cotton IPM, pp. 85-119. *In* R.E. Frisbie, K.M. El-Zik and L.T. Wilson (eds.), Integrated Pest Management System and Cotton Production. Wiley, New York.

Toxic Effect of Ethiopian Neem Oil on Larvae of Cattle Tick, *Rhipicephalus pulchellus* Gerstaeker.

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ABSTRACT

This study was conducted to test susceptibilities of cattle tick, *Rhipicephalus pulchellus* Gerstaeker after exposure to neem oil. The ultimate goal was to determine the bioacaricidal activity of Ethiopian neem extract under laboratory conditions for the control of *R. pulchellus* larvae. The LT_{50} values were determined for populations from each subsequent treatment by probit analysis and significant increases (chi-square test, *P>0.01*) occurred from one concentration to the next. There was approximately a 5.4-fold increase in the LT_{50} when concentration of 40% neem oil ($LT_{50} = 0.184$) was compared to 10% neem oil ($LT_{50} = 1.01$). In addition, serial concentrations of neem oil were applied and knock down concentration (KC_{50}) was obtained ($KC_{50} = 13.43$). Baseline information from these experiments will serve as a guide for future studies on susceptibilities of tick, *R. pulchellus* populations.

Key words: neem oil, cattle tick, bioacaricidal activity

INTRODUCTION

Ticks are important ectoparasites of domestic animals and affects 800 millions cattle and sheep around the world. They affect their hosts by damaging their hides and skins, reducing their growth rates and milk production and transmitting diseases organisms (Sutherest and Wilson, 1986). *Rhipicephalus pulchellus*, a three host tick species, has been recorded from an extremely wide ranges of domestic and wild animal especially ungulates (Walker, 1995). It is the commonest tick on domestic livestock, particularly cattle, sheep, goat and camels in some parts of eastern Ethiopia and northern Somalia (Pegram, 1976; Pegram *et al.*, 1981). Approximate counts made *in situ* on individual cattle in Borana District, Sidamo Province, Ethiopia, was approximately 100 ticks per animal infestation. (Walker *et al.*, 2000). In Somali region of Ethiopia

where this research was conducted, 90% of the tick were often obtained from cattle and camels compared to another pests.

Rhipicephalus pulchellus has been associated with wide variety of pathogenic organisms affecting both animals and men i.e., anaplasmosis, brucellosis, anthrax. *Trypanosoma theleria*, a parasitic organism, was found in the tissues and haemolymph of 19 out of 258 *R. pulchellus* adults from cattles in some provinces of Ethiopia i.e., Negeli Borana, Sidamo Province, Ethiopia (Burgdorfer *et al.,* 1973). Nairobi sheep disease virus was first reported to transmit experimentally by Lewis (1949). Pellegrini (1950) subsequently demonstrated the potential of trans-stadial and transovarial transmission of the virus through tick.

Acaricidal spray has been the commonest method of tick control in Africa since 1890.

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However, chemical control may provoque many problems such as environmental pollution, development of resistant tick strains and more expensive costs (Dipeolu and Ndungu, 1991). In view of the above problems, there has been an increasing interest in searching for alternative sustainable control methods of tick control in recent years. These include biological control by means of pathogens (Kaaya *et al.*, 1995).

Biological insecticides have proved as one of effective control. Several plants extract have been used and introduced in many areas. Extract of neem tree (*Azadirachta indica* A. Juss), a native of Burma and arid regions of the Indian sub continent, have been traditionally used by farmers in Asia and Africa to control insect pests of household, agricultural and medical importance. It has been reported that neem extract provides broad-spectrum control of over 200 species of phytophagous insects (Ascher, 1993) and they remain less toxic to natural enemies of insect pests than to the pests themselves (Schumutter, 1990).

Neem extract comprises a compound with diverse behavioral and physiological effects on insects, including feeding, deterrence, inhibition of growth and oviposition (Saxena, 1989; Schumutter, 1990). Although neem has been used for centuries for the control of household and agricultural pests, very limited researches were conducted for the controlling of livestock ticks.

The objective of this study was to determine the bioacaricidal activity of Ethiopian neem extract under laboratory conditions for the control of *R. pulchellus* larvae which is considered as a major livestock tick species in Somali region of Ethiopia.

MATERIALS AND METHODS

Rearing tick colony

The engorged females of *Rhipicephalus pulchellus* were collected from cattle in Jigjiga clinics and Jigjiga slaughter house in Somali region, eastern of Ethiopia. They were reared in glass tubes

in the incubator at $24\pm2°C$ and $70\pm5\%$ RH. Using the technique of Solomon and Kaaya (1998), the larvae were separated and kept in other tubes of further investigation.

Preparation of Ethiopian neem oil solution

Dry Ethiopian neem seed (*Azadirachta indica* A. Juss) were provided from Diredawa nursery center, 160 km east of Jigjiga. To obtain neem oil, neem seeds were crashed using the kornet vegetable machine. The powder of neem seeds were squeezed between the metals, which neem oil was obtained for further experiment. The neem oil was then checked for percentage azadirachtin by High Pressure Liquid Chromatography (HPLC).

Solution of neem oil, diluted with distilled water, at different concentrations ranging from 10-40% were tested against the larvae of *R. pulchellus* by topical application. Solution at each concentration was pipetted at 0.5 ml and was applied onto 15 larvae of one month old, while distilled water was applied as a control treatment. There were three replicates for each concentration. The treated ticks larvae were dried on filter paper and transferred to the covered petridish kept at room temperature of $24\pm2°C$ and $70\pm5\%$ RH. Their mortalities were continuously observed and recorded for a period of 24 hours. Ticks larvae which were unable to move at the end of each exposure period were considered as "dead". The mortality of tick was assessed and recorded at 30 min, 1 hr, 2 hr, 3 hr and 24 hours. The knockdown effects and LT_{50} values of each concentration were analysed by Probit Analysis.

RESULTS AND DISCUSSIONS

Toxic effect of Ethiopian neem oil on *Rhipicephalus pulchellus* larvae

Toxicity of Ethiopian neem oil against *R. pulchellus* was studied for the first time in Ethiopia. Dry neem seeds were crashed and the powder was squeezed between the two metals. From the total of

2 kg of dry neem seed, 3 ml of neem oil was obtained. Oil was then tested against one-month old of *R. pulchellus* larvae at the concentrations of 10, 20, 30 at 40%. Lethal Time-50 (LT_{50}) values for all four concentrations of neem oil are presented in Table 1.

LT_{50} values were ranged from 0.184 to 1.01. The smallest LT_{50} was obtained from neem oil concentration of 40% (0.184) whereas the highest was from neem at the concentration of 10% (1.01). No tick mortality was observed from the control during the investigation. Neem oil at 40% was the most effective and highest toxicity among the treated concentrations whereas neem at 10% showed the least toxicity (Table 1).

The slopes of regression line for test data from each neem concentration were computed, ranging from 0.504 to 2.36. The highest value was obtained from the neem concentration of 40% and the smallest slope was from the neem concentration of 10%. Small Chi square values indicated that the response of tick to neem oil in susceptibility test to follow completely a linear model (P>0.01).

This study implied that the acaricidal toxicity of Ethiopian neem oil varied directly with the concentration when tested against tick larvae. Higher concentration of neem oil produced high percent mortality than the lower concentration. However, neem oil at 30% was found to be the most effective and economically uses among the treated concentrations.

Ethiopian neem oil was investigated for the percentage of Azadirachtin with HPLC. Azadirachtins A and B at the level of 0.119% and 0.034% were detected from the sample of Ethiopian neem oil. Generally, Azadirachtin is non-toxic to tick when applied by topical application technique (Schmutter, 1990). It will produce toxicity effect only when ingested by the insects. Azadirachtin disrupts metamorphosis of insect and tick, hence they die without reproduction of new generation (Schmutter, 1990). However, the concentrations of Ethiopian neem oil used in this experiment were relatively high. All treated tick larvae were dead after exposing to 50% neem oil. Tick responded quickly to neem oil as indicative by the KC_{50} (13.43) and KC_{95} (78.91) values (Table 2).

Larval and nymphal stages of *R. pulchellus* can be easily controlled by using products of neem whereas the adult stage are not much sensitive to all employed dosages of the locally made neem products ($KC_{95} = 78.91$%). It is strongly recommended that the minimum dose of Ethiopian neem oil applied on cattle in the field should not be less than 20%. To facilitate in control approach, the concentration can be as much as 30%, depending on each economic problem of the farmer.

The main objective for this experiment was to find the most simple and effective method for controlling cattle tick in Ethiopia. Neem oil seems to be possible in controlling *R. pulchellus* larval in Ethiopia. It is also known that, tick control using

Table 1 Acaricidal toxicity (LT_{50}) of ethiopian neem oil against *Rhipicephalus pulchellus* larvae by topical application.

Treatment neem oil (%)	N*	LT_{50} (%) (level of confidence = .95)	Slope ± SE	Chi square
10	45	1.01 (0.198-2.190)	0.504 ± 0.155	1.6511
20	45	0.29 (0.00-1.007)	0.452 ± 0.196	0.1224
30	45	0.21 (0.179-0.426)	0.57 ± 0.485	0.4978
40	45	0.184 (0.00-0.36)	2.36 ± 1.0772	0.4211

N* = number of tick larvae used in each treatment.

Table 2 Knockdown concentration (KC) values of neem oil against *Rhipicephalus pulchellus* larvae by topical application.

Knock down level	KC values	95% confidential limit
KC-20	5.432	2.948-7.986
KC-50	13.43	9.478-17.016
KC-95	78.91	62.88-110.98

only acaricides is not an achievable goal under socioeconomic circumstance of Africa where most owners of livestock in the area are poor. Therefore, attempts should be made to search for other effective control methods, the application of neem oil for controlling ticks seems to be one of the most effective and more suitable under this circumstance. Even though neem found abundance in Ethiopia and the method of application as acaricidal property is not complicate, knowledge of formulation or ready made neem oil for commercial is scarce. Further detailed research must be done to determine the most effective and specific formulation of neem against ticks by overcoming all the limitations.

CONCLUSION

The engorged females of *Rhipicephalus pulchellus* were collected from cattle in Jigjiga, eastern of Ethiopia. They were reared in glass tubes in the incubator at 24±2ºC and 70±5% RH. Using the technique of Solomon and Kaaya (1998), the larvae were separated and kept in other tubes of further investigation.

Ethiopian neem seeds were crashed using the kornet vegetable machine. The powder of neem seeds were squeezed between the metals, which neem oil was obtained for further experiment and was checked for percentage azadirachtin by High Pressure Liquid Chromatography (HPLC).

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ACKNOWLEDGEMENT

LITERATURE CITED

Ascher, K.R.S. 1993. Non conventional insecticides effects of pesticide available from the neem tree, *Azadirachta indica*. Arch. Of insect biochem. Physiol. 22 : 433-449.

Burgdorfer, W., M.L. Scmidth, and H. Hoogstral. 1973. Detection of *Trypanosoma theileri* in Ethiopian cattle, Acta topica 30 : 340-346.

Dipeolu, O.O. and J.N. Ndungu. 1991. Acaricidal activity of "Kupetaba" a ground mixture of natural products against *Rhipicephalus appendiculatus*. Veterinary Parasitology. 38 : 327-338.

Kaaya, G.P., E.N. Mwangi, and M.M. Malanza. 1995. Acaricidal activity of *Margaritaria discoedea* plant extracts against the ticks *Rhipicephalus appendiculaltus* & *Hyalomma verigatum*. Int. J. Acarol. 21 : 123-129.

Lewis, E.A. 1949. Nairobi sheep disease, pp. 45-51. *In* Report of the veterinary department, Kenya, for 1947.

Pegram, R.G. 1976. Ticks (Acarina, Ixodidea) of the northern regions of the Somali Democratic Republic. Bulletin of Entomological Research. 66 : 345-363.

Pegram, R.G., H. Hoogstraal, and H.Y. Wassef. 1981. Ticks (Acari : Ixodidae) of Ethiopia. I, Distribution, ecology and host relationships of species infesting livestock, Bulletin of Entomological Research 71 : 339-359.

Pellegrini, D. 1950. La gastroenterite emorragica delle pecore. Esperimenti di transmissions col "*Rhipicephalus pulchellus*". Bulletino delle societa italiana di medicina e d'igeiene Tropicale 10 : 164-169.

Philip, C.B., H. Hoogstraal, R.J. Reiss-Gutfreund, and C.M. Clifford. 1966. Evidence of ricketsial diseases agents in tick from Ethiopia cattle. Bulletin of the World Health Organization (WHO) 35 : 127-131.

Richard, D. 1975. Study of the pathology of dromedary in Borana Awraja, Ethiopia, Thesis, I.E.M.V.T., Paris.

Saxena, K.N. 1989. Insect pest management & socioeconomic circumstances of small scale farmers for food crop production in western Kenya. A case study. Insect Sci. Appl. 10 : 446.

Schumutter, H. 1990. Properties & potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu. Rev. Entomol. 35 : 271-297.

Solomon, G. and G.P. Kaaya. 1998. Development reproductive capacity and survival of *Amblyomma varigatium* and *Boophilus decolaratus* in relation host resistance and climatic factors under different field conditions. Veterinary parasitology 75 : 241-253.

Sutherest, R.W. and L.J. Wilson. 1986. Tropical legumes and their ability to immobilize and kill cattle tick, pp. 185-194. *In* B.E. Juniper and T.R.E. South Wood (eds.). Insect and Plant Surface. Edaward and Arnold, London.

Walker, J.B., James, E. Reisans, and Ivan. 2000. The genus *Rhipicephalus* (Acari, Ixodidae). A guide to the brown ticks of the world. 643 pp.

Walker, J.B. 1995. *Rhipicephalus pulchellus* Gerstaeker. 1873. A discription of the larvae and the nymph with notes of the adults and on its biological. Parasitology 45 : 80-95.

Ileal and Faecal Amino Acids Digestibility of Some Tropical Feedstuffs in Growing Pigs

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ABSTRACT

To investigate apparent ileal and faecal digestibility of protein and amino acids in some tropical feedstuffs, six barrows, average initial body weight (BW) 25 kg, were fitted with a simple T-cannula at the distal ileum and fed six diets according to a 6 × 6 Latin square design. Three monodiets which three types of cereal, broken rice, rice bran and corn, as the protein source and three cornstarch- based diets using soybean meal, peanut meal and fishmeal as the protein sources and containing 12 % crude protein were used. Chromic oxide was included as a digestibility marker. After a 5 days adaptation period, faeces were collected for 3 days and followed with 12 hours digesta collection for 2 days in each experimental period. Apparent digestibility values over the total tract were found to be greater than values determined at the ileum, indicating a net disappearance of both nitrogen and amino acids in the hind gut. It was also observed that the apparent faecal and ileal digestibilities of nitrogen and amino acids in growing pigs was highest in soybean meal and ricebran showed the lowest values.

Key words: protein, amino acids, digestibility, pigs, tropical feedstuffs

INTRODUCTION

Formulation of diets based on digestible instead of total protein and amino acids content could decrease the nitrogen excretion from pigs. Because of the intense activity of microorganisms in large intestine, the digestibility overall of intestinal tract does not provide a good estimation of the individual amino acids digestibility (Lenis, 1992). It has been shown that the bacterial nitrogen in faeces is about 62-76 % of the total nitrogen. This part of protein nitrogen is not avaiable to animals, because the digestion and absorption of external protein are completely at the end of small intestine in pigs (Mason, 1984). Therefore, ileal digestibility is more suitable for pig to predict the quality of dietary protein. Recent studies have clearly shown

that the ileal digestible values are highly correlated to growth as well as the protein deposition (Rademacher *et al*., 1996) In consideration of the variation of quality and nutrient composition among the feedstuffs, the determination of digestibility of protein and amino acids in the tropical feedstuffs was studied for feed formulation in Thailand.

MATERIALS AND METHODS

Six barrows (Landrace × Large white × Pietrain), averaging about 25 kg, were surgically fitted with a simple T-cannula at the distal ileum according to procedures adapted from Karsten (1995). After surgery, the animals were individually housed in 2 m × 2 m concrete floored pens. After a 14-day recovery period, six diets consisted of three

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monodiets (Table 1) containing cereal or a cereal by product (corn, broken rice and ricebran) as the protein source and three semi-purified diets based on cornstarch and sucrose containing three sources of protein (soybean meal, peanut meal and fishmeal) to provide 12 % crude protein were tested according to a 6×6 latin square design. Chromic oxide 0.25 % was included as a marker for the determination of digestibility. Animals were fed twice daily at a level of 100 g/kgBW$^{0.75}$ /day. Diets were mixed with an equal portion of water and feed. Water was provided *ad libitum*. The average of initial and final body weights for the collection period were 42.9 ± 4.5 and 78.7 ± 9.3 kg, respectively.

Each experimental period was consisted of 10 days. After a 5 days adaption period, the faeces were collected for 3 d, followed by a 2 days ileal digesta collection between feeding period (12 hours). Ileal digesta were collected in soft plastic bags attached to the barrel of the cannula. The samples were immediately frozen at –20°C. Faeces samples were collected twice daily and stored at –20°C until analysis.

Faeces and digesta samples were dried and ground through a 1-mm mesh screen. Analyses of the nutrient component of the diets were carried out according to AOAC (1984) methods. Chromic oxide concentration was determined as described by Bolin *et al.* (1952). Amino acids were analyzed using HPLC and the samples were hydrolyzed and derivatived with phenylisothiocyanate (PITC) to form phenylthiocarbamyl amino acids as described by Waters (1989) and detected by UV detector with 254 nm. Tryptophan content was not determined in this study. All of data were shown in mean values ± standard deviation.

RESULTS

Apparent faecal digestibility

The protein and amino acid composition of the experimental feedstuffs is presented in Table 2

Table 1 Composition of the experimental diets (% in the diet).

Composition	Diet I	Diet II	Diet III	Diet IV	Diet V	Diet VI
Corn	95.54	-	-	-	-	-
Broken rice	-	95.45	-	-	-	-
Ricebran	-	-	96.35	-	-	-
Cornstarch	-	-	-	57.6	56.6	66.55
Soybean meal	-	-	-	26.0	-	-
Peanut meal	-	-	-	-	27.0	-
Fishmeal	-	-	-	-	-	20.7
Sucrose	-	-	-	10.0	10.0	10.0
Ricebran oil	-	-	-	2.0	2.0	2.0
Dicalcium phosphate	3.11	3.45	1.20	3.30	3.30	-
CaCO$_3$	0.25	-	1.35	-	-	-
NaCl	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin /mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50
Chromic oxide	0.25	0.25	0.25	0.25	0.25	0.25

1: vitamin/mineral premix per kg diet : 16,000 IU Vit. A; 1,340 IU Vit. D$_3$; 20 mg Vit. E; 1.4 mg menadione; 2 mg Thiamin; 4 mg Riboflavin; 2.6 mg Pyridoxine; 0.024 mg Cobalamin; 10 mg d-Ca-pantothenate; 20 mg Niacin; 0.5 mg Folic acid; 0.2 mg D-Biotin; 300 mg Choline-chloride ; 0.2 mg Se; 80 mg Fe; 50 mg Mn; 100 mg Zn; 150 mg Cu; 0.2 mg Co; 0.5 mg I

and the apparent faecal digestibilities of nitrogen and amino acids are shown in Table 3. Ricebran had the lowest digestibility of protein and amino acids compared to other protein sources, whereas the values between other feedstuffs showed only little difference. Of the indispensable amino acids, arginine and leucine in all protein sources showed the highest digestibility and the digestibility of threonine normally was lowest.

Apparent ileal digestibility

The digestibilities of protein and amino acids at the end of small intestine showed greater difference between feedstuffs (Table 4). For the values measured over the digestive tract, the digestible protein and amino acids from pigs fed with ricebran as the protein source were lower than

the other feedstuffs in this study. The digestibility of arginine and leucine was high in all of six feedstuffs, whereas threonine and lysine showed low digestibility at the end of small intestine. Of the nonessential amino acids, glutamic acid had the highest value and glycine, proline and cystine showed the low digestibilities.

DISCUSSION

The faecal and ileal digestibility of protein and amino acids in cereal and cereal-by products agreed well with those from literatures, such as in corn, broken rice and ricebran (Yin *et al*., 1993). Some variation of the digestibility from the same feedstuff may be caused by the altering of the relative amount of each of four major proteins in

Table 2 Protein and amino acid composition of feedstuffs (in %).

Items	Corn	Broken rice	Ricebran	Soymeal	Peanut meal	Fishmeal meal
Crude protein	8.30	6.87	13.1	46.1	44.4	56.7
Essential amino acids						
Arginine	0.44	0.56	1.00	3.28	4.81	3.39
Histidine	0.26	0.18	0.32	1.26	1.20	1.38
Isoleucine	0.27	0.23	0.46	1.94	1.28	2.09
Leucine	1.06	0.53	0.87	3.07	2.84	4.08
Lysine	0.28	0.25	0.58	2.75	1.47	3.83
Methionine	0.19	0.17	0.26	0.61	0.49	1.44
Phenylalanine	0.42	0.37	0.61	2.24	2.17	2.28
Threonine	0.33	0.27	0.50	1.77	1.17	2.35
Valine	0.47	0.39	0.64	1.95	1.99	2.43
Nonessential amino acids						
Alanine	0.65	0.36	0.76	2.03	1.81	3.43
Aspartic acid	0.58	0.62	1.16	4.65	4.92	4.60
Cystine	0.19	0.17	0.24	0.64	0.58	0.53
Glutamic acid	1.55	1.15	1.63	8.08	8.15	7.28
Glycine	0.32	0.31	0.57	2.19	2.31	4.02
Proline	0.79	0.34	0.66	2.23	1.87	2.47
Serine	0.38	0.33	0.59	2.21	2.17	2.20
Tyrosine	0.32	0.28	0.38	1.67	1.79	1.67

cereal (albumin, globulins, prolamines and glutelines) which could be depended on the variety of grain, fertilizer application and environmental conditions (Mosenthin *et al.*, 1997). The nutrient composition in cereal by product especially showed the great variable according to the processing techniques. Warren and Farrell (1989) reported that the crude protein content in ricebran from the different part of Australia varied from 134 to 173 g/ kg DM depend on the content of broken rice and husk and could be affected on the variation of digestibility. Comparing between cereal and cereal by-product, the faecal and ileal digestibilities of protein and amino acids were as follows : broken rice > corn > ricebran. The higher content of crude fiber in ricebran could be due to the increasing digesta movement and the reducing digestion time (Schutte *et al.*, 1992).

As the higher fiber content might stimulated the secretion of endogenous protein, Boisen and Moughan (1996) reported that the endogenous protein of pigs averaged 10-15 g/kg DM-intake by feeding free protein diet and increased to 20-40 g/ kg DM-intake by feeding diet being consisted of fiber and antinutritive substances. The fraction of fiber also affected the digestibility. Mariscal (1992) reported that NDF (neutral detergent fiber) had more negative effect on the ileal digestibility than ADF (acid detergent fiber). Ricebran contained high content of NDF (256 g/kg DM ; Warren and Farrell, 1989). Therefore, the digestibility of amino acids in ricebran was low.

Table 3 Apparent faecal digestibilities of nitrogen and amino acids of feedstuffs.

Item	Apparent faecal digestibility (in %)					
	Corn	Broken rice	Ricebran	Soybean meal	Peanut meal	Fishmeal
Nitrogen	83.9 ±1.9	88.5 ±3.5	67.8±7.3	86.6±3.4	86.2±2.0	81.4±2.7
Essential amino acids						
Arginine	88.8±1.3	91.8±1.2	85.4±2.8	90.9±2.5	91.5±1.2	86.5±0.6
Histidine	84.7±3.4	86.1±1.1	70.6±4.1	88.6±1.4	86.0±2.0	84.1±1.5
Isoleucine	83.8±1.8	88.0±1.1	69.8±6.1	86.0±2.4	85.0±0.9	86.6±2.3
Leucine	91.1±1.6	90.8±0.8	69.0±7.0	86.5±1.6	86.9±2.6	86.8±2.1
Lysine	84.8±3.3	87.6±2.1	74.5±5.8	90.1±1.7	82.8±1.0	87.5±1.2
Methionine	84.5±1.3	87.9±0.9	79.8±3.8	83.6±3.2	82.9±3.5	85.7±3.5
Phenylalanine	88.4±1.4	89.9±1.1	72.4±5.0	88.1±2.9	88.2±2.1	83.8±2.5
Threonine	79.2±2.3	87.5±2.4	65.5±7.4	85.8±2.8	81.3±2.3	82.9±4.3
Valine	83.0±3.1	87.4±1.1	72.3±6.3	84.8±3.4	85.0±1.1	82.4±1.8
Nonessential amino acids						
Alanine	88.0±2.1	87.2±1.5	74.4±5.3	83.3±3.6	85.0±1.2	84.5±3.1
Aspartic acid	85.5±2.9	89.3±1.8	69.2±6.3	88.5±2.0	92.5±0.8	88.7±1.8
Cystine	84.8±1.8	85.1±1.9	64.9±7.4	84.6±2.6	84.6±1.1	83.5±1.7
Glutamic acid	90.7±0.9	91.5±1.0	81.6±3.9	91.4±1.6	91.7±0.3	89.3±1.7
Glycine	80.8±3.0	87.8±0.8	76.9±5.0	84.5±2.9	85.3±1.6	82.1±1.2
Proline	86.3±1.9	86.6±3.0	72.6±6.1	87.3±1.6	85.1±3.2	82.8±1.6
Serine	87.0±1.8	86.8±1.6	72.9±4.2	88.2±2.5	88.5±2.7	82.7±2.4
Tyrosine	83.7±1.6	87.1±1.4	72.8±5.7	87.3±2.7	86.9±2.5	83.2±3.1

The apparent ileal nitrogen and amino acids digestibilities in high-protein feedstuffs (soybean meal, peanut meal and fishmeal) reported in this study agreed with the values, summerized by Mosenthin *et al*. (1997). There was considerable variation in the apparent ileal amino acid digestibilities, especially in fishmeal. A review of those studied revealed that the digestibilities were determined in diets with varying amino acids content. Other factors included inherent factors in different samples, processing techniques, and cannulation methods (Sauer and Ozimek, 1986).

The faecal digestibilities of nitrogen and amino acids were consistently higher than when measured at the end of the small intestine, indicating a loss of nitrogenous components in the caecum and colon, affected by the microbial fermentation. Amino acids that were the great component of endogenous protein and had the low ileal digestibility, such as threonine, glycine and proline, the losses of these amino acids in the hindgut were high. This showed that the endogenous protein was intensively digested by microflora in caecum and colon (Sauer *et al*., 1991). The nitrogen from these amino acids was lost mostly as ammonia which can be absorbed and excreted as urea in the urine (Just *et al*., 1981). In the case of methionine, the ileal digestibility was not different from faecal digestibility and in some cases, the post ileal digestibility showed negative value. This might reflect the net synthesis by transformation of cystine to methionine in the large intestine (Weerden *et al*.,

Table 4 Apparent ileal digestibilities of nitrogen and amino acids of feedstuffs.

Item	Apparent ileal digestibility (in %)					
	Corn	Broken rice	Ricebran	Soybean meal	Peanut meal	Fishmeal
Nitrogen	73.8 ±2.5	78.4 ±1.5	61.1±6.2	82.2±1.5	78.0±1.3	72.4±4.4
Essential amino acids						
Arginine	84.4±1.0	84.9±2.0	82.1±2.3	86.8±1.1	88.0±1.0	84.2±0.5
Histidine	74.2±2.8	77.8±3.4	63.5±2.9	80.3±1.8	79.2±1.4	82.4±1.5
Isoleucine	79.3±2.6	83.0±1.2	67.0±7.4	84.6±3.0	83.1±2.0	83.4±2.1
Leucine	83.4±1.2	84.6±2.8	67.2±7.0	84.2±1.4	82.1±1.9	84.8±1.6
Lysine	76.9±3.2	79.9±1.6	71.8±3.4	86.3±0.9	78.6±1.4	84.3±1.6
Methionine	81.6±1.1	85.7±1.9	78.7±5.8	82.2±2.8	83.9±2.2	83.9±3.2
Phenylalanine	83.7±2.5	82.3±1.9	69.7±5.4	83.9±2.1	83.3±1.4	80.4±1.7
Threonine	63.4±3.5	74.4±3.2	56.6±8.7	75.6±4.6	68.8±3.3	78.9±3.8
Valine	78.2±3.0	81.0±2.5	67.8±5.8	82.0±2.5	81.2±1.4	80.2±1.6
Nonessential amino acids						
Alanine	82.6±1.6	80.4±2.4	69.1±6.0	80.0±2.7	80.3±1.7	82.0±3.7
Aspartic acid	77.9±4.3	82.7±2.0	67.5±6.2	84.8±1.3	87.1±1.2	83.2±3.1
Cystine	75.8±2.1	76.3±1.7	59.8±7.4	73.8±2.4	77.3±2.1	73.0±2.4
Glutamic acid	86.4±1.5	88.3±1.8	79.5±4.0	88.9±1.4	88.7±1.3	87.3±1.4
Glycine	56.4±4.1	72.7±2.9	67.4±5.2	74.6±3.5	76.7±1.9	77.3±1.8
Proline	61.3±3.0	60.4±6.1	54.7±6.5	78.2±3.3	75.6±2.4	78.1±2.4
Serine	80.1±2.6	79.8±2.9	69.8±3.9	84.4±2.4	83.2±2.4	77.6±3.9
Tyrosine	80.8±1.5	82.2±2.6	70.2±6.3	82.2±1.6	83.5±1.8	80.4±2.6

1980).

The ileal analysis method was found to be more sensitive than faecal method for determining amino acids digestibility in feedstuffs for pigs. However, the number of the tropical feedstuffs that have been carried in this study were limited. To formulate diet on the basis of digestible, as opposed to total, amino acids supply for the pig production in Thailand, more studies using diets with a wider array of feedstuffs are necessary.

CONCLUSION

Measured by the faecal analysis method, there was only a slight difference in the digestibility of the amino acids in the six feedstuffs tested. Greater differences were found by the ileal analysis method. Of all six tropical feedstuffs investigated, the ileal and the faecal digestibility of crude protein and amino acids were highest in soybean meal and lowest in ricebran.

LITERATURE CITED

A.O.A.C. 1984. Official Methods of Analysis of the Association of Official Analytical Chemists. 14th ed. Association of Official Analytical Chemists, Washington, DC. 1141 p.

Boisen, S. and P.J. Moughan. 1996. Dietary influences on endogenous ileal protein and amino acid loss in the pig. A review. Acta Agric. Scand. 46 : 154-164.

Bolin, D.W., R.P. King, and E.W. Klosterman. 1952. A simplified method for the determination of chromic oxide (Cr_2O_3) when used as an index substance. Science. 116 : 634-635.

Just, A., H. Jorgensen, and J.A. Fernandez. 1981. The digestive capacity of the caecum-colon and the value of the nitrogen absorbed from the hind gut for protein synthesis in pigs. Br. J. Nutr. 46 : 209-219.

Karsten, D. 1995. Praecaekale Verdaulichkeit von 15N- und Homoarginin markierten Milchproteinen beim juvenilen und adulten Miniaturschwein. Inaugural-Dissertation Fachberich Veterinaermedizin der Justus-Liebig-Universiaet. Giessen, Germany. 107 p.

Lenis, N.P. 1992. Digestible amino acids for pigs: Assessment of requirements on ileal digestible basis. Pig News and Information. 13 : 31N-39N.

Mariscal Landin, G. 1992. Facteurs de variation de l' utilisation digestive des acides amins chez le proc. Thaese d' Universitae Rennes, france

Mason, V.C. 1984. Metabolism of nitrogenous compounds in the large gut. Proc. Nutr. Soc. 43 : 45.

Mosenthin, R., M. Rademacher, and W.C. Sauer. 1997. Zur scheinbaren praezaekalen Verdaulichkeit von Aminosaeuren in Futtermittteln fuer Schweine. Uebers. Tierernaehrg. 25 : 41-85.

Rademacher, M., R. Mosenthin, and W.C. Sauer. 1996. Die Anwendung des Konzeptes der praecaecalen Aminosaeurenverdaulichkeit in der Rationsgestaltung von Mastschweinen. 4 Tagung "Schweine-und Gefluegelernaehrung", Halle/Saale, 133-136.

Sauer, W.C. and L. Ozimek. 1986. Digestibility of amino acids in swine: Results and their practical application: A review. Livest. Prod. Sci. 15 : 367-388.

Sauer, W.C., R. Mosenthin., F. Ahrens, and L.A. den Hartog. 1991. The effect of source of fiber on ileal and faecal amino acid digestibility and bacterial nitrogen excretion in growing pigs. J. Anim. Sci. 69 : 4070-4077.

Schutte, J.B., J. de Jong., E.J. van Weenden, and S. Tamminga. 1992. Nutritional implications of L-arabinose in pigs. Br. J. Nutr. 68 : 195-207.

Warren, B.E. and D.J. Farrell. 1989. The nutritive value of full fat and defatted australian ricebran. 1. Chemical composition. Anim. Feed Sci. Technol. 27 : 219-228.

Waters. 1989. Pico-Tag Amino Acid Analysis System Operator's Manual. Millipore

Corporation, Milford, Massachusetts.123 p.

Weerden, E.J.van., P. Slump, and J. Huisman. 1980. Amino acid digestion in different parts of the intestinal tract of pigs, pp. 207-214. *In* H.J Oslage and K. Rohr (eds.). Proceedings of the 3rd EAAP Symposium on Protein Metabolism and Nutrition, Vol. I, Braunschweig, F.R. Germany

Yin, Y. L., R.L. Huang., H.Y. Zhang., C.M. Chen., T.J. Li, and Y.F. Pan. 1993. Nutritive value of feedstuffs and diets for pigs. 1. Chemical composition, apparent ileal and faecal digestibilities. Anim. Feed Sci. Technol. 44 : 1-27.

Stimulation of Shell Regeneration by Crude Extract of Subesophaqeal Ganglionic Mass in Giant African Snails, *Achatina fulica* (Bowdich)

Viyada Seehabutr

ABSTRACT

The distribution of cells that stained positively with paraldehyde fuchsin (PAF) and negatively with chrome-hematoxylin phloxine (CH) in the subesophageal ganglionic mass of *Achatina fulica* has been mapped. The PAF-positive cells occur in the visceral ganglion, each cell contains electron-dense elementary granules of 1,300 Angstrom in diameter. From serial section, it showed that the PAF-positive cells or neurosecretory cells (NSC) sent axons into the intestinal nerve. The CH-negative cells occurred in the right parietal ganglion, each cell contained electron-dense elementary granules of 1,370 Angstrom in diameter. From serial sections, it showed that CH-negative cells sent axons into the pallial nerve.

PAF-positive and CH-negative materials were depleted from cells in 24 hr after shell removal and reappeared by 72 h. Shell regeneration apparently completed within 15 days. Moreover, the subesophageal ganglionic mass homogenate (SGH) had some effects on shell regeneration which suggested that the PAF-positive and CH-negative cells were the neurosecretory cells that control shell regeneration.

Key words: neurosecretory cells, paraldehyde-fuchsin, chrome-hematoxylin phloxine, pallial nerve, shell regeneration, subesophageal ganglionic mass homogenate

INTRODUCTION

Achatina fulica is a land snail. It belongs to the phylum Mollusca, class Gastropoda, subclass Pulmonata, order Stylommatophora, family Achatinidae. These land snails are plentiful in the tropical countries with high rainfall. The shell of *A. fulica* consists of 7 to 12 whorls, with moderately swollen body whorl and a sharply conical spire, which is distinctly narrowed but scarcely drawn out at the apex. The snail has no gill and operculum, but the mantle cavity serves as a lung. It has two pairs of retractile tentacles, with eyes at the tips of posterior tentacles.

The nervous system of *A. fulica* consists of 13 ganglia; a pair of buccal ganglia, a pair of cerebral ganglia, a pair of tentacular ganglia and a subesophageal ganglionic mass. The subesophageal ganglionic mass is a mass of nervous tissues lies under the esophagus and columellar muscle. It is composed of seven ganglia; two pleural ganglia, two parietal ganglia, two pedal ganglia and a single visceral ganglion.

Seehabutr (1992) reported that there are neurosecretory cells in procerebrum of cerebral ganglia of *A. fulica*. The crude extract of these ganglia has effects on oogenesis in the ovotestis of *A. fulica*. In this experiment, the possible role of neurosecretory cells on shell regeneration in *A. fulica* has been examined.

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MATERIAL AND METHODS

1. **Histochemical preparation for morphological study of the neurosecretory cells by light micro-scopy**

1.1 Experimental animals and paraffin embedment

Mature *A. fulica* with the shell height of 7 cm were used in this experiment. They were collected from the field during the rainy season. Subesophageal ganglionic mass were removed from snails and processed for serial paraffin sections. The 6 um thick serial sections were then submitted for further staining. Chrome- hematoxylin phoxine and paraldehyde-fuchsin were used to identify the neurosecretory cells in subesophageal ganglionic mass.

1.2 Chrome-hematoxylin phloxine staining method (Gomori, 1941).

Subesophageal ganglionic mass were fixed in Bouin's fluid and processed through paraffin section at 6 um thick. The section were tested for about 1 min with a solution containing about 0.3% each of potassium permanganate and sulfuric acid, then decolorized with a 2 to 5% solution of sodium bisulfite. After staining with hematoxylin solution, the sections were counterstained with 0.5% aqueous solution of phloxine (B) (Gomori, 1941).

1.3 Paraldehyde-fuchsin staining method (Gomori, 1950).

The paraffin sections (6 um thick) were deparaffinized, hydrated and oxidized in Gomori's fluid. After staining in paraldehyde-fuchsin solution the sections were counterstained in Halmi's mixture (Cameron and Steele, 1959). Then, the sections were examined under the bright field of microscope.

2. **Histological preparation for ultrastructural study of the neurosecretory cells by transmission electron microscopy**

The specimens of subesophageal ganglionic mass were embedded in araldite. The plastic blocks of tissue were sectioned using glass knives and stained with uranyl acetate, and counterstained with lead citrate. Then the samples were examined using Hitachi H-300 electron microscope.

3. **Subesophageal ganglionic mass homogenate preparation**

Adult *A. fulica* (about 5 whorls, 5-6 cm long and 15-20 g in weight) were collected from the wild during May to October, 1999. These snails were anesthetized with nembutal for 30 minutes. The subesophageal ganglionic mass were dissected, cut into small pieces and frozen on dry ice before extraction. Subsequently, this ganglionic tissue was ground in normal saline; pH 8.5 using hand homogenizer (Pelluet and Lane, 1961) (the one milliliter of saline was used to extract 2-3 ganglionic mass). The sample was then centrifuged at about 3,000 *g* for 20 minutes. The clear supernatant was collected.

4. **Experimental design**

Adult snails (14-18 g in weight) were collected from the wild and kept in the containers for 7 days before testing. The snails were divided into 3 groups (30 snails /group); the control group, the sham-operated group (received 10 um of normal saline per snail once a week for 4 weeks by oral method), the experimental group [received subesophageal ganglionic mass homogenate (2 mass/snail) once a week for 4 weeks by oral method]. To initiate shell regeneration, a piece of 1 cm? was removed at the growing edge.

All the snails were kept in the plastic container at 22°C under 12 Light : 12 Dark photoperiod. The diet was supplied *ad libitum.* The weight and shell regeneration of each snail were recorded every week throughout the experiment.

RESULTS

Distribution of neurosecretory cells

The distribution of neurosecretory cells in the subesophageal ganglionic mass of *A. fulica* that

stained with PAF and CH were shown in Figures 6-7.

PAF-positive cells were found in the visceral ganglion. There were about 15 cells with an average individual diameter of 50-70 um lying posterior to the visceral-parietal connective tissue (Figure 1). At the ultrastructural level these cells were found to contain electron dense granules of 1,300 Angstrom in diameter (Figure 2). The cell bodies contain prominent rough endoplasmic reticulum and accumulations of glycogen and numerous golgi complexes. Large dense bodies resembling lysosomes varied in abundance.

CH-negative cells were found in the right parietal ganglion. There were about 30-36 cells with an average individual diameter of 60-80 um lying in the anterior part of the ganglion (Figure 1). At the ultrastructural level these cells were found to contain electron dense granules of 1,370 Angstrom in diameter (Figure 3). The organelles in the cells bodies were the same as PAF-positive cells of visceral ganglion.

Changes in the neurosecretory cells during shell regeneration

After removing a large segment of the shell from the growing edge, regeneration appeared to be completed within 15 days. Twenty-four hours after

Figure 2 A-B-C TEM Micrograph of visceral ganglion

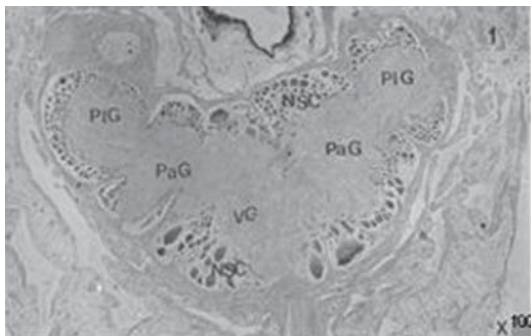|		
N	=	nucleus
HC	=	heterochromatin
EC	=	euchromatin
Mi	=	mitochondria
Lcy	=	lysosome
RER	=	rough endoplasmic reticulum
Mt	=	microtubule
arrow	=	electron dense elementary granule

Figure 1 subesophageal ganglionic mass
        PIG = pleural ganglion
        PaG = parietal ganglion
        VG = visceral ganglion
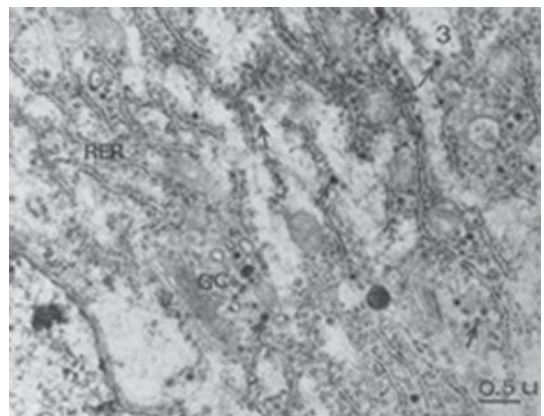

Figure 3 TEM Micrograph of parietal ganglion
        RER = rough endoplasmic reticulum
        GC   = golgi complex
        arrow = electron dense elementary granule

shell removal the cell bodies in visceral and parietal ganglia showed reduced staining reduction, or none at all, with PAF and CH, whereas the right pallial and the intestinal nerves showed dark staining with PAF and CH (Figure 5-6). However the stained material begins to reappear in the cell bodies of visceral and parietal ganglia about 72 hours after shell removal (Figure 6, 7).

Effect of subesophageal ganglionic mass homogenate (SGH) on shell regeneration

One week after treating with normal saline and SGH, the width of regenerated shell of control group, normal saline group and SGH group were 2.3 mm, 2.94 mm and 3.5 mm, respectively.

In the second week, the average width of regenerated shell of the control group, normal saline group and SGH group were 8.2 mm, 7.1 and 9.2 mm, respectively.

The shell regeneration of all groups was complete in the third week after treatment. However the shell regeneration of SGH treated group was prior and thicker (0.35 mm) than those of the control group (0.28 mm) and normal saline groups (0.27 mm).

DISCUSSION

A correlation has been demonstrated between changes in the histological appearance of neurosecretory cells in subesophageal ganglionic mass (VG, PaG) and the early stages of the shell repair after damage in *A. fulica*. This correlation suggests a possible role of neurosecretory material in the control of shell repair. This finding agrees with the work of Dillaman *et al. (1976)* on the neurosecretory cells in visceral ganglion having some effects on shell regeneration.

Saleuddin (1975) reported that the mantle edge gland, which is responsible for laying down the shell material, is supplied by neurosecretory axons and it is possible that the axons that run in the right pallial nerve may innervate the mantle edge
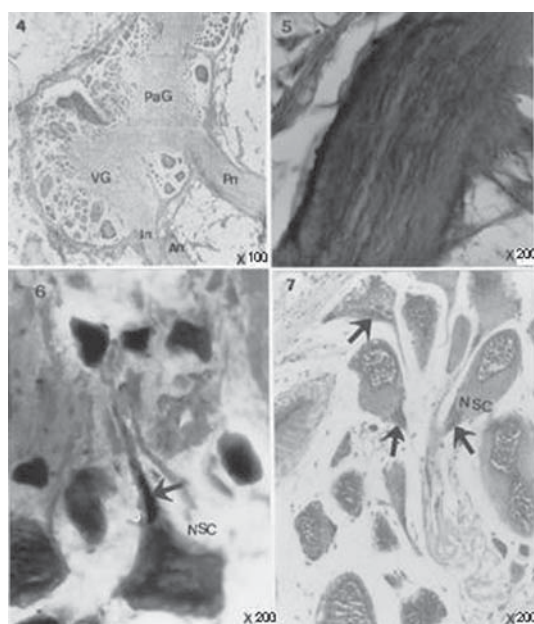
Figure 4 subesophageal ganglionic mass
   Pag = parietal ganglion
   VG = visceral ganglion
   In = intestinal narve
   An = anal nerve
   Pn = pallial nerve
Figure 5 Intestinal nerve
   arrow - axons of neurosecretory cells
Figure 6 Neurosecretory cells (PAF-positive material)
Figure 7 Neurosecretory cells (CH-negative cell) of parietal ganglion
   arrow = neurosecretory material (CH-negative material)

directly (Kerkut and Walker, 1975). So, it could be concluded that the neurosecretory cells in PaG might control the shell regeneration in *A. fulica*. Moreover, there are some neurosecretory cells in visceral ganglion and their axons accumulated neurosecretory material running into the intestinal nerve. This finding agrees with the research of Simpson (1969) that most of the neurosecretory axons from the visceral ganglion run in the intestinal nerve, terminating partly in the aorta and partly in

the heart.

Information on the effect of SGH on shell regeneration of *A. fulica* could lead us to determine the location and action of NSC in PaG and VG.

CONCLUSION

The cells that stained positively with paraldehyde fuchsin (PAF) could be found in the visceral ganglion and the cells that stained negatively with chrome-hematoxylin phloxine (CH) could be found in the right parietal ganglion. The subesophageal ganglionic mass homogenate has some effects on shell regeneration of *A. fulica* after administration by oral method.

ACKNOWLEDGEMENTS

LITERATURE CITED

Cameron, M.L. and J.E. Steele. 1959. Simplified aldehyde-fuchsin staining of neurosecretory cell. Stain Technol. 31 : 265.

Dillaman, R.M., A.S.M. Saleuddin, and G.M. Jones. 1976. Neurosecretion and shell regeneration in *Helisoma duryi* (Mollusca:Pulnata). Can. J. Zool. 54 : 1771-1778.

Gomori, G. 1941. Observation with differential stains on human Islets of Langerhans. Am. J. Path. 17 : 395-406.

Gomori, G.1950. Aldehyde-fuchsin: A new stain for elastic tissue. Am. J. Clin. Path. 20 : 665-666.

Kerkut, G.A. and R.J. Walker. 1975. Pulmonates; Nervous System, Eye and Statocyst. Vol. 1. London, New York, Sanfrancisco: Academic Press. 245 p.

Pelluet, D. and N.J. Lane. 1961. The relation between neurosecretion and cell differentiation in the ovotestis of slugs (Gastropoda: Pulmonata). Can. J. Zool. 39 : 691-805.

Saleuddin, A. S.M. 1975. An electron microscopic study on the formation of the periostracum in *Helisoma* (Mollusca). Calcif. Tissue Res. 18 : 297-310.

Simpson, L. 1969. Morphological studies of possible neuroendocrine structures in *Helisoma tenue* (Gastropoda:Pulmonata). Z. Zellforsch. 102 : 570-593.

Seehabutr, V. 1992. Structure and function of neuroendocrine organs in giant African snails *Achatina fulica* (Bowdich). Ph.D. thesis, Mahidol University, Bangkok.

Hematology, Morphology and Ultrastructure of Blood Cells and Blood Parasites from Puff-faced Watersnakes (*Homalopsis buccata*)

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ABSTRACT

Blood samples of 45 puff-faced watersnakes (*Homalopsis buccata*) in the Queen Saovabha Memorial Institute were collected from ventral caudal vein for both basic hematology and light microscopic, scanning and transmission electron microscopic features of blood cells. Seventeen samples (37.8%) were positive for hematozoa infections. The single infection of *Hepatozoon* sp., trypanosome and *Haemogregarina* sp. was found in 4, 4 and 5 snakes respectively. The other four snakes were infected by both trypanosome and *Hepatozoon* sp. There were no significant differences of all hematological value between the hematozoa-negative and the hematozoa-positive snakes except fibrinogen concentration which was found higher in the negative group. Lymphocytes were the most commonly observed leukocytes and average 6-8 μm in diameter. Azurophils were the second most commonly observed leukocytes, average 10-17 μm in diameter and might play a major role in eliminating the trypanosome. Heterophils were the largest leukocytes, average 16-19 μm in diameter and the third commonly observed leukocytes. Eosinophils usually were medium-sized cells, average 10-14 μm in diameter but in some occasion the very large cells were also detected. Basophils were smaller than heterophils and eosinophils. Scanning electron microscopy revealed the membrane surfaces of normal and abnormal erythrocytes, *Hepatozoon* sp. infected erythrocytes, thrombocytes, eosinophil and trypanosomes. Transmission electron microscopy revealed the organelles within azurophil, eosinophil, heterophil and trypanosome.

Key words: Electronmicroscopy, *Haemogregarina*, Hematology, *Hepatozoon*, *Homalopsis buccata*, puff-faced watersnakes, trypanosome

INTRODUCTION

Puff-faced or mask-faced watersnake (*Homalopsis buccata*) is immediately identified by the large broad head and a white, mask-like pattern on the top of the head. It consumes fish and frogs. Its habitat is commonly found in most of Southeast Asia (Cox *et al*., 1998). The Queen Saovabha Memorial Institute (QSMI) had initiated a captive breeding program since 1994 to supply healthy snakes for venom and antivenom production. These venomous snakes prey on mice and occasionally on puff-faced watersnakes. The venomous snakes were highly infected with *Hepatozoon* sp. (Salakij *et al*., 2001). Reptile blood cell morphologic characteristics are heterogeneous. Variations in cell characteristics and cell populations were existed between species within the order Squamata (Alleman *et al*., 1999).

The purpose of this study was to obtain the hematological data, characterization of blood cells

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and compare if the hematozoas in the puff-faced water snakes were the same as those found in the venomous snakes.

MATERIALS AND METHODS

Blood samples of 45 puff-faced watersnakes were collected from ventral caudal vein during January to March 2000. Blood smears were prepared immediately and air-dried. They were stained with Wright's and Wright-Giemsa stained for white blood cell differentiation and hemoparasite examination. The collected blood samples were kept immediately in EDTA, immediately stored at 4°C and processed within 2 hours.

The packed cell volume (PCV) were determined by microcapillary technique. The total solids were measured using a Atago®SPR-N refractometer (Japan). Fibrinogen was calculated as the difference between the total solids before and after incubation for 3 minutes at 56°C and recentrifugation (Jain, 1986). The total red blood cell count (RBC), were determined manually with the improved Neubauer counting chamber after the blood was diluted 200 times with the Natt and Herrick's solution (Natt and Herrick, 1952). The total number of the RBC was counted in the five red blood cell squares of the center large square of the chamber in duplicate. The duplicates were averaged for agreement within 15% difference and multiplied by 10,000 to calculated the number per microlitre (Campbell, 1986). The leukocyte count (WBC) was determined in the same counting chamber as the RBC count except the leukocytes were counted in duplicate from four large squares of the chamber at 40X magnification. The leukocyte neuclei were stained blue whereas the thrombocyte neuclei were unstained or very pale blue. The duplicates were averaged for agreement within 15% difference and multiplied by 500 to calculate the number per microlitre. The hemoglobin concentration (Hb) was determined by the cyanomethemoglobin method which free RBC neuclei were removed by the centrifugation before reading the absorbance (Campbell, 1986).

Blood smears were fixed in methanol and stained with Wright-Giemsa (WG) stain (Benjamin, 1978) for determination of differential leukocyte count, identification of hematozoa infection and morphological evaluation of all blood cells. Grading of *Hepatozoon* sp. and *Haemogregarina* sp. was quantitated by the number of infected erythrocytes as described elsewhere (Salakij *et al.*, 2001). A minimum of 200 leukocytes were counted for differential leukocyte determination. For comparison, blood smear from 5 puff-faced watersnakes were stained with one step Wright's staining method that did not required methanol fixation prior to staining stain (Benjamin, 1978).

Blood samples were also prepared for reticulocyte count by staining with new methylene blue using wet preparation (Benjamin, 1978). The percentage of reticulocytes presented in 1,000 erythrocytes was determined. The reticulocytes that contained distinct aggregated reticulum were described as aggregate reticulocytes whereas punctate reticulocytes contained a few small dots (Jain, 1986).

For each parameter obtained, data from hematozoa-negative and positive were calculated for means, variances and standard error using SPSS® for window ™ (Norusis, 1993). Significant difference between means were determined using an independent sample T-test model.

For scanning electron microscopy (SEM), a drop of blood were fixed using 1.5% glutaraldehyde (GA) in 0.1 M phosphate buffer (PB) at 4°C for 24 hr. Specimens were dehydrated through a graded acetone series. Gold-coated smears were examined under Jeol JSM-35CF scanning electron microscopy.

For transmission electron microscopy (TEM), buffy coats were fixed in 2.5% GA (PB) for 24 hr and postfixed in 1% osmium tetroxide. Specimens were dehydrated through a graded acetone series and embedded in Spurr's epoxy

resin. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined using Jeol 1200Ex TEM.

RESULTS

Seventeen samples (37.8%) were positive for hematozoa infections. The single infection was found in thirteen snakes including: *Hepatozoon* sp., trypanosome and *Haemogregarina* sp. The other four snakes (8.9%) were mixed infection between trypanosome and *Hepatozoon* sp. (Table 1). There were no significant differences of all hematological values between the hematozoa-negative and the positive groups except fibrinogen concentration which was higher in the negative group (Table 2).

Erythrocytes were homogeneous in color but moderately anisocytosis (Figure 1, 2, 3). Cytoplasmic holes were detected in less than 1% erythrocytes (Figure 3c). The other shapes of abnormal erythrocytes were seldom detected (Figure 3b, 3c). *Hepatozoon*– and *Haemogregarina*-infected erythrocytes were larger than those non-infected ones (Figure 2b, 2d, 3e). By SEM, erythrocytes were ellipsoidal lacking the doming appearance in the site of the nucleus (Figure 3a, 3b, 3c).

Thrombocytes were elongate and approximately half the size of mature erythrocytes. Under the Wright's stain, cytoplasm was slightly basophilic (Figure 1i) compared with the unstained cytoplasm with azurophil granules in Wright-Giemsa stain (Figure 1a). Thrombocytes were easily differentiated from lymphocytes by the characteristic perinuclear and cytoplasmic vacuolation (Figure 1a, 1i). By SEM, their membranes were more irregular than those of erythrocytes (Figure 3a).

Leukocytes of puff-faced water snakes were categorized into 6 groups; azurophil, heterophil, eosinophil, basophil, lymphocyte and monocyte. For comparison, the blood smears stained with one step Wright's stain provided staining quality for identification of all blood cell type but in Wright's stain the erythrocytes stained more basophilic (Figure 1h, 1i).

Lymphocytes in puff-faced watersnakes were the most prevalent circulating cells (Table 2). They were small, well differentiated and averaged 6-8 µm in diameter (Figure 1a).

Azurophils were the second most commonly observed leukocytes, which contained fine indistinct azurophilic granules. They were round and 10-17 µm in diameter. The nuclei were round to irregular with clump chromatin and located centrally to eccentric (Figure 1b, 1d). Ultrastucturally, they contained numerous membrane-bound granules, some mitochondria and rough endoplasmic reticulum (Figure 4b). Phagocytosis of trypanosome by azurophils was detected only by TEM (Figure 4b). The number of monocytes is very rare and their characters were similar to mammalian monocytes (Figure 1d).

Heterophils were the largest of the leukocytes and average 16-19 µm in diameter. They contained large numbers of irregular shape, dull eosinophilic granules (Figure 1c). By Wright's stain, heterophil granules were easily seen by reddish-orange bright granules (Figure 1h). Ultrastructurally, heterophils contained pleomorphic population of large granules with variable electron density (Figure 4d).

Eosinophils contained numerous round and light blue granules that often occluded visualization of the nucleus (Figure 1e, 1h). They usually were medium-sized cells (10-14 µm in diameter) but in some cases very large cells were also detected (Figure 1f, 1i). By Wright's stain, eosinophil granules were stained dark blue when compared to the heterophils (Figure 1h). By SEM, their granule contour was bulging showing the custard apple-liked appearance (Figure 3d). Ultrastructurally, eosinophils contained homogeneous electron density granules with some cytoplasmic projections (Figure 4c). The eosinophil number were very high both in the negative and positive groups (Table 2).

Basophils were very low, average 9-12 µm in diameter and were slightly smaller than

Table 1 Number and percentage of hematozoa-negative and positive puff-faced watersnakes which was subgrouped according to sex.

	Male	Female	Total	%
Negative	15	13	28	62.2
Hepatozoon sp.	2	2	4	8.9
Trypanosoma sp.	4	0	4	8.9
Trypanosoma sp. and *Hepatozoon* sp.	3	1	4	8.9
Haemogregarina sp.	5	0	5	11.1
Total	29	16	45	100
%	64.4	35.6	100	

Table 2 Comparative hematology (mean ± SE) between hematozoa-negative and positive puff-faced watersnakes.

	Hematozoa-negative	Hematozoa-positive
Number	28	17
PCV (%)	19.6 ± 1.2	22.3 ± 1.6
Hb (g/dl)	6.34 ± 0.38	6.99 ± 0.46
RBC ($\times 10^6/\mu l$)	0.530 ± 0.046	0.618 ± 0.068
WBC ($\times 10^3/\mu l$)	12.11 ± 1.00	11.95 ± 1.78
Azurophils ($\times 10^3/\mu l$)	4.22 ± 0.48	3.96 ± 0.82
Heterophils ($\times 10^3/\mu l$)	1.91 ± 0.26	1.31 ± 0.17
Basophils ($\times 10^3/\mu l$)	0.005 ± 0.003	0.02 ± 0.01
Eosinophils ($\times 10^3/\mu l$)	0.69 ± 0.10	0.70 ± 0.14
Lymphocytes ($\times 10^3/\mu l$)	5.16 ± 0.70	5.93 ± 1.26
Monocytes ($\times 10^3/\mu l$)	0.08 ± 0.03	0.03 ± 0.02
Azurophils (%)	35.8 ± 3.0	32.5 ± 3.9
Heterophils (%)	15.3 ± 1.6	13.1 ± 2.1
Basophils (%)	0.07 ± 0.05	0.2 ± 0.09
Eosinophils (%)	6.5 ± 0.9	6.2 ± 0.9
Lymphocytes (%)	41.3 ± 2.8	47.5 ± 4.3
Monocytes (%)	0.8 ± 0.4	0.4 ± 0.2
PP (g/dl)	5.7 ± 0.30	5.6 ± 0.40
Fibrinogen (mg/dl)	142.8 ± 20.8*	64.7 ± 17.0*
Agg. Reticulocytes (%)	0.74 ± 0.30	3.27 ± 1.68
Punct. Reticulocytes (%)	4.11 ± 1.32	9.61 ± 2.83

* Significant difference at $p<0.05$.

eosinophils (Figure 1g). They contained small dark purple staining metachromatic granules that obscure the lobed nucleus.

Trypomastigote form of trypanosomes were large, broad body width (Figure 2a). By SEM, they were often in a cluster (Figure 3f). Ultrastructurally, they contained nucleus, very large mitochondria, abundant ribosomes (Figure 4b), some dense and multivesicular bodies (Figure 4a). The gamonts of *Haemogregarina* sp. were easily defined from those of the *Hepaozoon* sp. by their very large-size and more granularity (Figure 2b). There were two kinds of *Hepaozoon* gamonts; the small (Figure 2c) and the large gamonts (Figure 2d). Both *Hepaozoon* and *Haemogregarina* gamonts were resided in the cytoplasm of enlarged erythrocytes (Figure 3e) and displaced the nucleus (Figure 2b, 2c). Some gamonts were free from erythrocytes within their
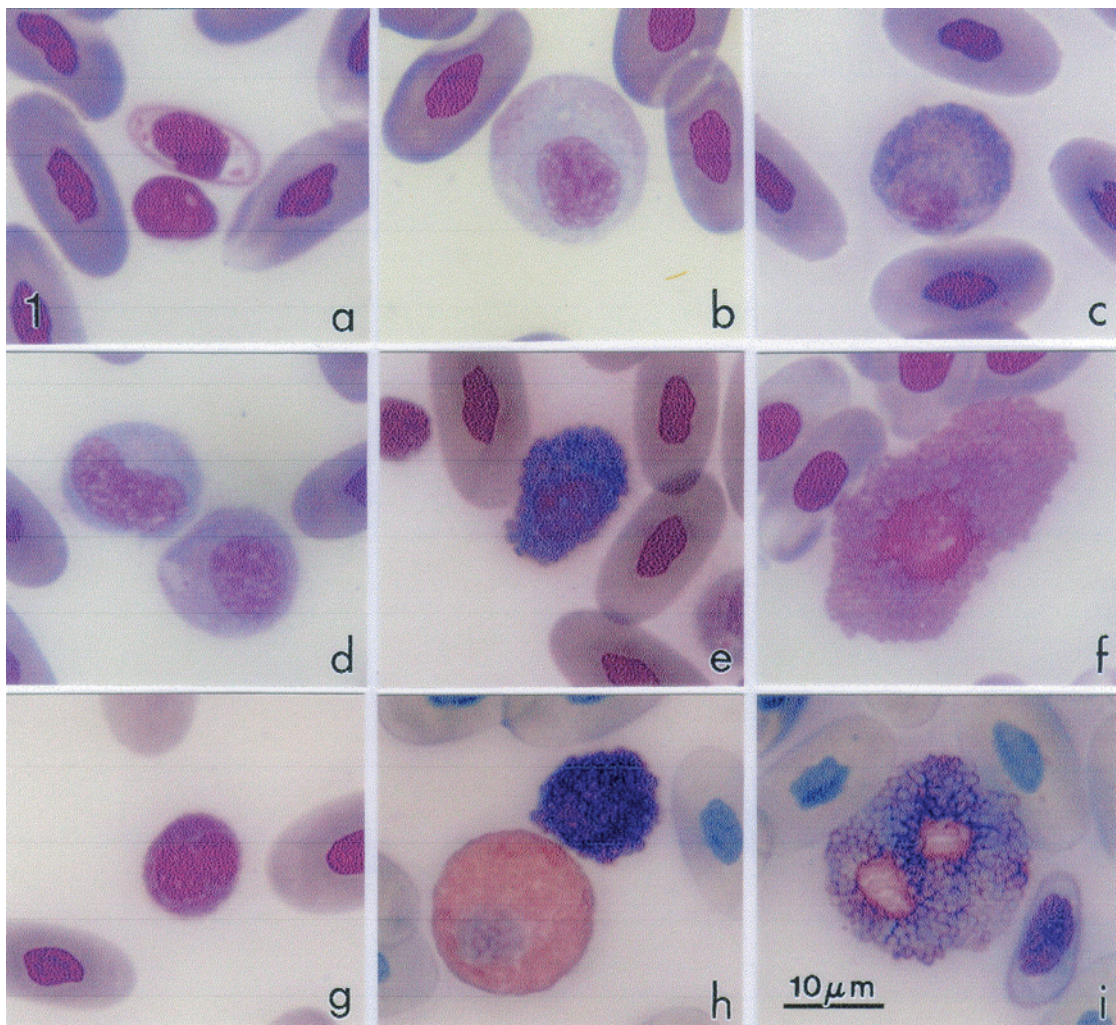
Figure 1 Blood cells in puff-faced watersnakes (a) a lymphocyte (lower cell) and a thrombocyte (b) an azurophil, (c) a heterophil, (d) a monocyte (left cell) and an azurophil, (e) an eosinophil, (f) a large eosinophil, (g) a basophil, Wright-Giemsa stain, (h) a 17 μm heterophil and an 14 μm eosinophil. Wright's stain, (i) a large eosinophil, Wright's stain.

parasitophorous vacuole membrane (Figure 3b).

DISCUSSION

The incidence of hematozoa infection in puff-faced watersnakes were as high as the other snakes in Queen Saovabha Memorial Institute (Salakij *et al.*, 2001). Some hematological values were different from the normal hematologic parameters for reptile (Mader, 2000) such as the PCV was lower whist the total WBC and the plasma protein was higher than the reference (Mader, 2000). This study also revealed that hematozoa parasitism of puff-faced watersnakes erythrocytes had no effect on anemia since there was no significant difference of all erythrocyte parameters. These results support the finding that no clinical disease was demonstrated in parasitized snakes (Campbell, 1986).

Lymphocytes in puff-faced watersnakes were the most prevalent circulating cells like those in King cobra (Salakij *et al.*, 2002) and the other snakes (Mader, 2000). Some researchers characterize azurophils as monocytes with azurophilic granules (Campbell, 1986). The finding of phagocytosing trypanosome by azurophil in TEM suggested that they may play a major role in elimination of trypanosome. This finding was observed only by TEM may because of the close
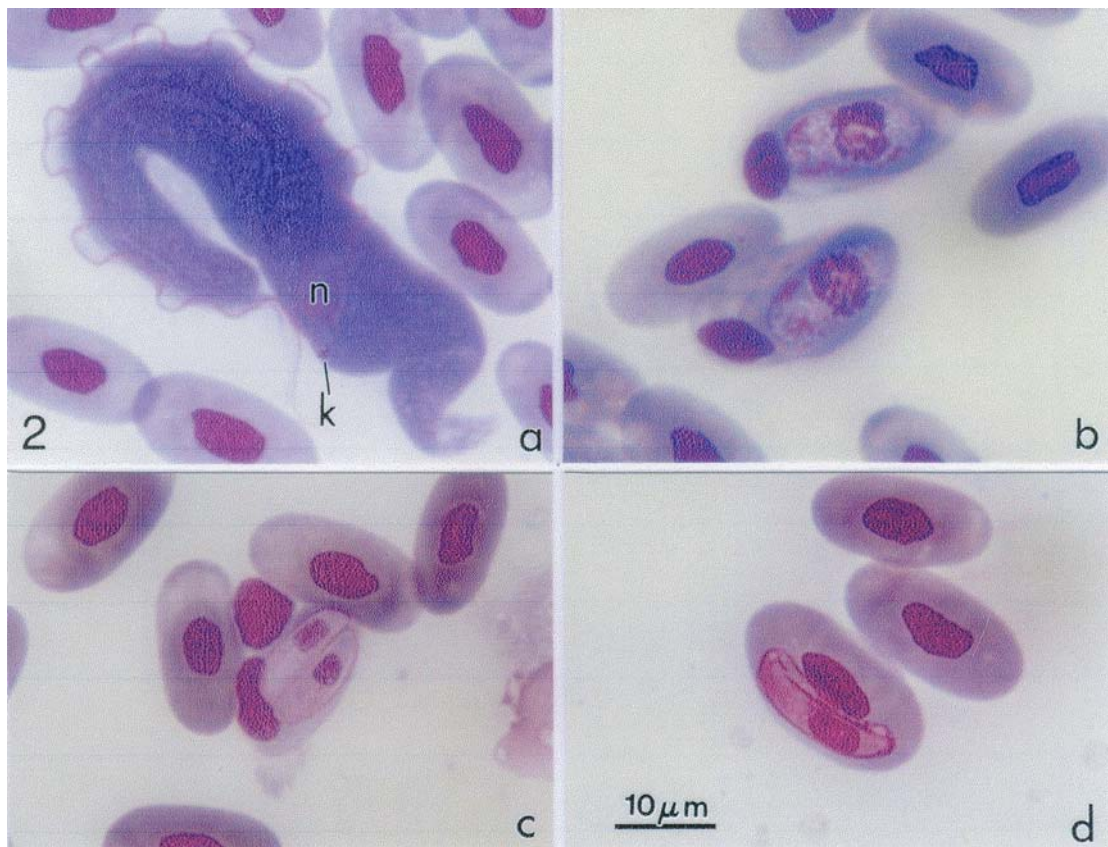
Figure 2 Hematozoa found in puff-faced watersnakes (a) trypomastigote of *Trypanosoma* sp. showing nucleus (n) and kinetoplast (k), (b) gamont of *Haemogregarina* sp, (c) two small gamonts of *Hepatozoon* sp., Note the displacement of the erythrocyte nucleus (d) large and curve gamont of *Hepatozoon* sp., Wright-Giemsa stain.

contact of the azurophils and the trypanosomes in the buffy coat before they were fixed with glutaraldehyde.

It is difficult to differentiate eosinophils from basophils in WG stained smears because of the bluish coloration of their granules. They were identifed more easily on Wright's stained preparation. The eosinophil granule characteristic in puff-faced watersnakes was similar to those of iguanas and psittacines (Hawkey and Dannett, 1989) which they contained dark purple staining metachromatic granules that obscure the unlobed nucleus. The large-sized eosinophils found in puff-faced watersnakes should be the characteristic of eosinophil in snakes which were larger than those of the other reptiles (Mader, 2000).
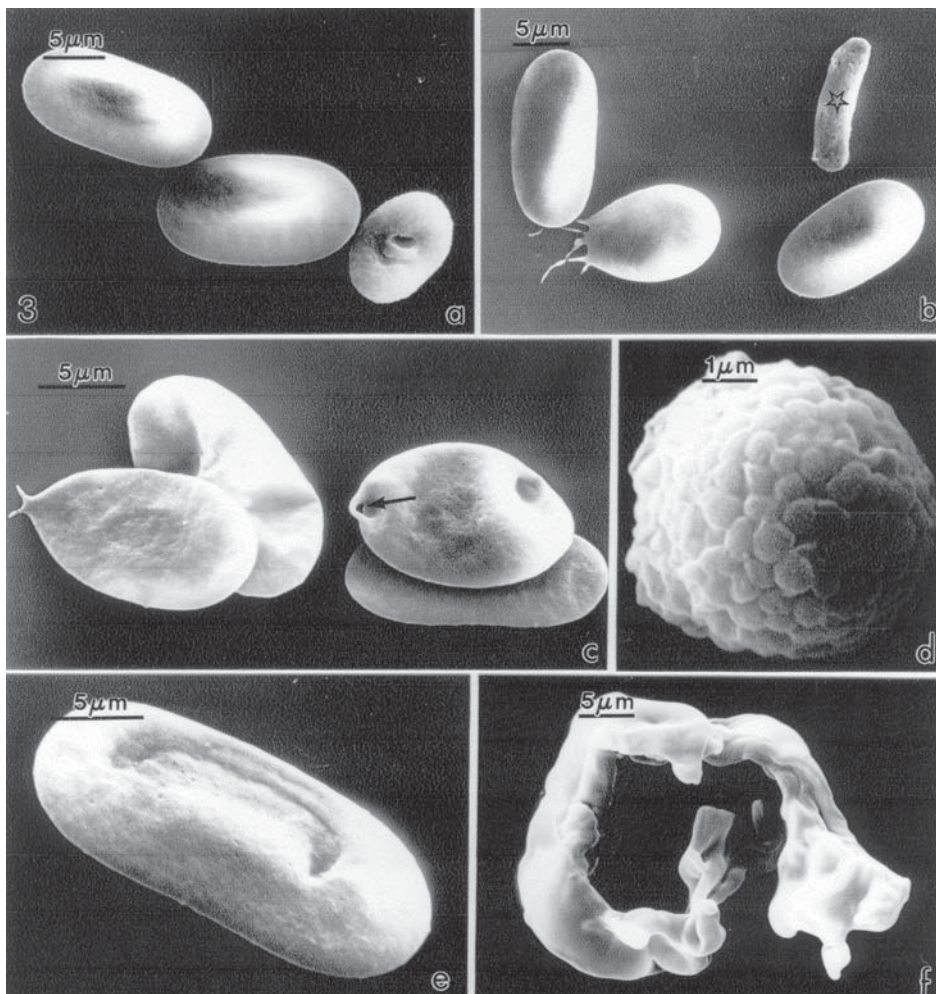
Figure 3 Scanning electron photomicrographs of (a) two normal erythrocytes and a thrombocyte, (b) euglenoil-shaped erythrocyte and parasitophorous vacuole membrane containing gamont of *Hapatozoon* sp. (star), (c) abnormal erythrocytes showing double appendages and cytoplasmic hole (arrow), (d) an eosinophil showing custard apple-like appearance of granule contour, (e) an enlarged erythrocyte containing *Hepatozoon* gamont, (f) a cluster of trypanosomes.

The high number of eosinophils in the negative and positive groups (Table 2) may be influenced by parasitic stimuli or other stimuli (Mader, 2000). The finding of eosinophils in puff-faced watersnake confirm the existence of these leukocytes in snakes eventhough they were not identified in eastern diamondback rattlesnakes (Alleman *et al*., 1999).

Trypomastigote form of trypanosomes in puff-faced watersnakes was different from *Trypanosoma hydrae* in broad-band watersnake from Louisiana (Chia and Miller, 1984). Trypanosomes were detected only in puff-faced and rainbow watersnakes of the QSMI (Salakij *et al*., 2001).
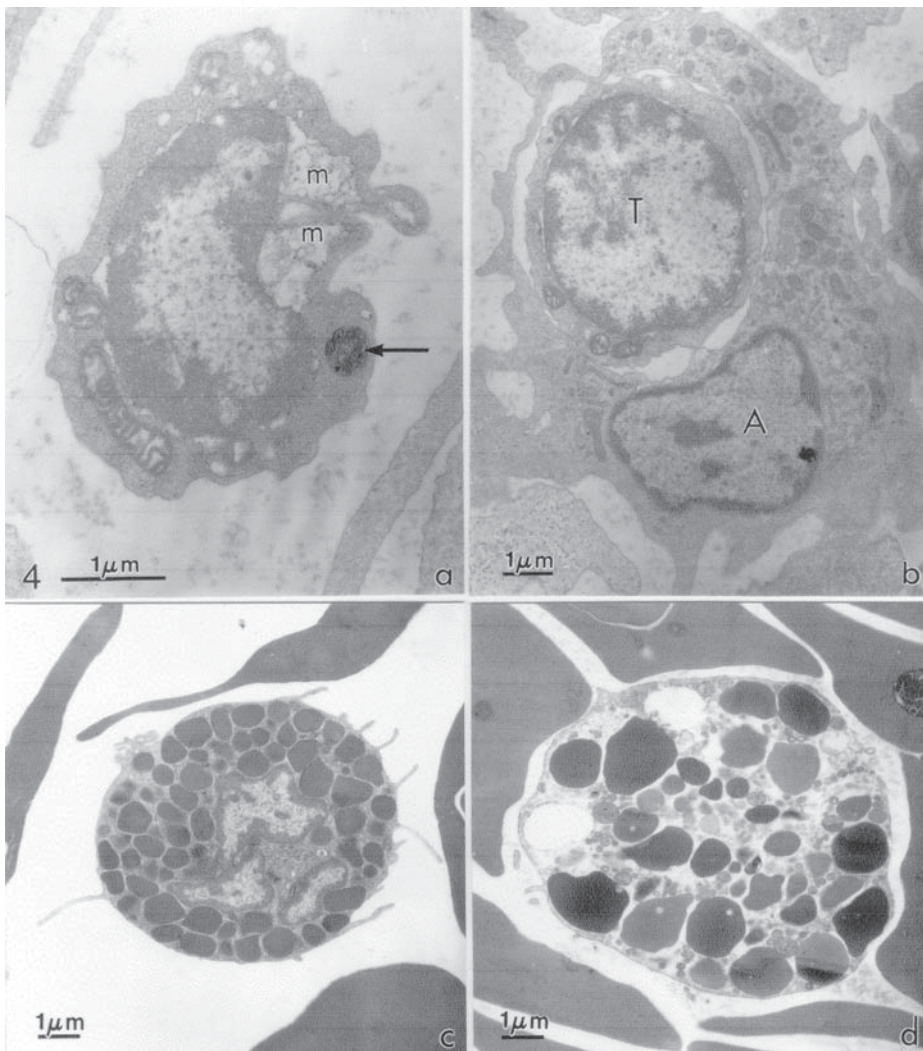
Figure 4 Transmission electron photomicrographs of (a) cross-section of a trypanosome containing nucleus, mitochondria, multivesicular bodies (m) and dense granular granule (arrow), (b) an azurophil (A) pseudopodia is surrounding a trypanosome (T), (c) an eosinophil with homogeneous granules and cytoplasmic process, (d) a heterophil with vacuoles and heterogeneous electron density granules.

The small gamonts of *Hepatozoon* sp. were similar to those found in the banded krait (*Bangarus fasciatus*) of the QSMI (Salakij *et al*., 2001). The large gamonts found in puff-faced watersnakes were referred as medium-sized gamonts when compared with the larger gamonts found in mangrove snakes and mangrove pit vipers of the QSMI (Salakij *et al*., 2001). *Haemogregarina* sp. was found not only in watersnakes but also in Burmese python, mangrove snakes and rainbow watersnakes of the QSMI (Salakij *et al*., 2001). These three kinds of snakes were not fed on puff-faced watersnakes so they were not transmitted by eating.

This study provides more information on the hematology, morphology and ultrastructure of blood cells in puff-faced watersnakes. This may be benificial for further study and related research.

ACKNOWLEDGMENTS

LITERATURE CITED

Alleman, A. R., E. R. Jacobson, and R. E. Raskin. 1999. Morphologic, cytochemical staining and ultrstructural characteristics of blood cells from eastern diamonback rattlesnakes (*Crotalus adamanteus*). Am. J. Vet. Res. 60 : 507-514.

Benjamin, M. M. 1978. Outline of Veterinary Clinical Pathology. 3rd ed. The Iowa State University Press, Ames. 351p.

Campbell T. W. 1986. Clinical Pathology, pp. 248-257. *In* D. R. Mader (ed.). Reptile Medicine and Surgery. WB Saunders, Philadelphia.

Chia, N. M. and J. H. Miller. 1984. Morphological and Developmental Studies of the Snake Trypanosome *Trypanosoma hydrae* Ayala,

Atkinson, Vakalis, 1983 in Experimentally Infected Hosts and in Culture. J. Protozool. 31 : 352-356.

Cox, M. J., P. P. van Dijk, J. Nabhitabhata and K. Thirakhupt. 1998. A Photographic Guide to Snakes and Other Reptiles of Thailand and Southeast Asia. ASIA BOOKS. Bangkok, 144 p.

Hawkey, C. M. and T. B. Dannett. 1989. A Color Atlas of Comparative Veterinary Haematology. Wolfe Publishing Limited. Ipswick. 192 p.

Jain, N. C. 1986. Schalm's Veterinary Hematology. 4th ed. Lea and Febiger. Philadelphia, 1221p.

Mader, D. R. 2000. Normal Hematology of Reptiles, pp. 1126-1132. *In* B. E., Feldman, J. G. Zinkl and N. C. Jain (eds.) Schalm's Veterinary Hematology. 5th ed. Lippincott Williams and Wilkins, Philadelphia.

Natt. M. P. and C. A. Herrick. 1952. A new blood diluent for counting erythrocytes and leukocytes of the chicken. Poult. Sci. 31 : 735-738.

Norusis, M. J. 1993. SPSS® for Window™ Base System User's Guide Release 6.0.SPSS inc., Chicago, Ill. 828 p.

Salakij, J., C. Salakij, N. Narkkong, L. Chanhome, N. Rochanapat, and P. Suthunmapinunta. 2001. Hematozoa of snakes in Queen Saovabha Memorial Institute. Kasetsart J (Nat Sci*) 35 : 149-156.

Salakij, C., J. Salakij, S. Apibal, N. Narkkong, L. Chanhome, N. Rochanapat, and P. Suthun-mapinunta. 2002. Hematology, morphology, cytochemical staining and ultrastructural characteristics of blood cells in King cobras (*Ophiophagus hannah*). Vet. Clin. Path. 31 : 116-126.

Functional Snack Food

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ABSTRACT

The direct-expanded functional snack food could be produced using a formula being composed of 70% corn grit, 10.5% soy protein isolate, 4% full-fat soy flour, 10% inactivated full-fat rice bran, seasoning with barbecue flavor, as well as vitamins and iodine added to be accepted by the consumer test (120 persons) at the 7- point level of medium-like (9-point hedonic scale). The products showed 2.88 expansion ratio, 0.16 g/cm^3 density, 73.96 newton hardness and 1.23 crispiness (Df). The chemical compositions showed that the barbecue flavor functional snack food contained 15.78% protein, 14.07% fat, 4.49% ash, 2.02% crude fiber and 5.86% dietary fiber. The nutritive values of this product calculated at 30 g/serving as Recommended Daily Intake (RDI) were 9.46% protein, 7.03% dietary fiber, 278.67% vitamin B_1, 188.82% vitamin B_2 and 31.4% iodine. The shelf-life stability was determined by packing the products in 2 types of packaging: thin and thick metalized polyethylene terephthalate (metalized PET), then stored at varied temperature (35°C and 55°C) for 8 weeks. The results showed that the high temperature (55°C) caused the physical properties, texture and taste-panel acceptance change more than the other. Thin metalized PET was the most suitable packaging to keep products within 8 weeks at 35°C. Moisture content (3.72 to 4.87%) and a_w (0.25 to 0.32) were slightly increased. Thiobarbituric acid increased (0.20 to 3.24 mg/1000g). Hardness was not significantly different ($P > 0.05$) (84.52 to 90.65 newton), whereas Df slightly decreased (1.24 to 1.13). The chemical compositions of 8-week snack stored at 35°C as dry basis showed no significantly different values for protein (16.06%) and crude fiber (1.51%) from the control samples but had some different values for fat (9.81%), ash (4.11%) carbohydrate (68.51%) and dietary fiber (8.35%). The taste panel accepted the product similar to normal in the range of acceptance (score 8-9).

Key words: snack food, functional food, soy protein, rice bran

INTRODUCTION

In general, snack foods were made from mainly cereal as base ingredients, which provided mostly energy from carbohydrates and fat. The protein content was about 3.3-8.3% depending on other ingredients added. The frequency of snack consumption of children aged 7-18 years old in Bangkok was found to be 51.3% consuming everyday (Sinthavalai, 1984). Kosayothin (1996) reported that the market size of snacks was greatly increased to 5,820 million baht and 40% of the market was shared by extruded (or direct-expanded) snack.

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This project was aimed to improve the nutritional value of the direct expanded snack to be the functional snack food for children. The main improvement was protein content from soy protein isolate and dietary fiber from full fat rice bran.

MATERIAL AND METHODS

Materials

Corn grit (30-50 mesh) was supplied by Thai Maize Products Ltd. Soy protein isolate (PROFAM 974) was obtained from Heinz Win Chance Ltd. Full fat soy flour was received from The Royal Project (Doi Kham). Full fat rice bran was supplied by CP Product Ltd. For longer keeping quality, rice bran was stabilized by extrusion cooking with twin-screw extruder at 130°C. Calcium carbonate (food grade) was supplied by Thai Food and Chemical Ltd. VITACEL® (wheat fiber) was purchased from Rama Production Co., Ltd. Sugar and soybean oil were purchased locally.

Extrusion process

The weighed raw materials were thoroughly mixed by a mixer (Telegram bear mixer) before transferred to a co-rotating twin screw extruder (ZE 25x33D, Hermann Berstorff Laboratory). The extruder was composed of 7 connecting barrels and a 25 mm. Thick die with the diameter of 3.00 mm. The length : diameter ratio was 870 : 25. The temperatures in the barrel No. 1-7 and 9 (at die) were 30, 35, 45, 95, 135, 155, 130 and 120°C, respectively. The mixed raw material were fed into the extruder at the rate of 385 ± 10 g/min. Moisture content of the feed was adjusted to $16 \pm 0.5\%$ (wet basis) by injecting an ambient temperature water. Screw speed was 300 rpm (Boonyasirikool and Charunuch, 1997). The adjustable die face cutter with one blade was operated at 300 rpm. The melting temperature was 156-158°C. The extrudate were dried at 80°C for 15 min in an electric oven. The dried products were allowed to cool to room temperature and immediately packed airtight in plastic bags and stored at room temperature.

Proximate composition determinations

Moisture, protein, fat, crude fiber, ash and dietary fiber of corn grit, soy protein isolate, full fat soy flour and full fat rice bran were determined in duplicate by AOAC (1990).

Physical properties determinations

Expansion ratio (ER) of the extrudates were examined by applying a micrometer to measure the diameter of a cylindrical shape sample. ER was defined as the ratio of a sample diameter to a diameter of the dies (Boonyasirikool and Charunuch, 1997).

Bulk density was modified from Boonyasirikool and Charunuch (1997) by filling a 100-mL graduated cylinder and determine the weight per volume (g/cm^3).

Texture analysis of the extrudates were characterized by using compressive measurements, which was carried out on a TA.XT2 texture analyzer with a compressing probe P_{100} (100 mm. dia. cylinder aluminum). The instrument settings were as follows: pre-test speed 5.0 mm/s; test speed 10.0 mm/s; post-test speed 10.0 mm/s;compression distance 50% of sample height. Five measurements were performed on each sample. The maximum compression was defined as the sample hardness. The fractal dimension value was defined as the sample crispiness (Stable Micro System, 1993).

Sensory evaluation

Samples from each formula were coated with oil and barbecue seasoning in the appropriate ratio (extrudate : vegetable oil : barbecue seasoning ; 81 : 8.5 : 10.5). The barbecue flavor snacks were evaluated by 24 panels of the graduate students in Food Science program, Kasetsart University. 9-point hedonic scale (1 = extremely dislike to 9 = extremely like) was used to determine color, odor, flavor, texture and total preference. The SAS software was applied for statistical analysis of

ANOVA and Duncan's multiple range test of difference between formula at 95% level of confidence.

Storage stability test

The 30-g barbecue flavor snack food was placed in 2 types of packaging; thin (PET12 / MCPP25) and thick (OPP20/PE18/MPET 12/ PE 23) metalized polyethylene terephthalate (metalized PET). Samples were stored at 35°C and 55°C for 8 weeks and removed to determine physico-chemical changes, textural characteristics and sensory evaluation.

Physico-chemical changes of samples were evaluated by moisture content (AOAC, 1990) and a_w (Thermoconstanter, model HUMIDAT-TH2, NOVASINA) measurements. Lipid oxidation was evaluated by thiobabituric acid (TBA) determinations (Yu and Sinnuhuber, 1957).

Textural characteristics were determined by using compressive measurements, which was carried out on a TA.XT2 texture analyzer. The maximum compression was defined as the sample hardness. The fractal dimension value was defined as the sample crispiness (Stable Micro System, 1993)

Sensory evaluation of the functional snack food was determined by 10-trained panels at weekly interval during a period of 8 weeks. Panels were asked to give their opinions about off-flavor, color, barbecue flavor and crispiness against control

samples stored in a dark cabinet at -18°C (Reilly and Man, 1994).

Statistical analysis

Data were analyzed using SAS data analysis software release 6.12 TSO20 Licensed to Louisiana State University. Analysis of variance and Duncan's multiple range test at (P = 0.05) were used to determine differences between treatments.

RESULTS AND DISCUSSION

Chemical compositions of raw materials (corn grits, soy protein isolate, full fat soy flour and full fat rice bran) were determined (Table 1).

The proximate analysis of four raw materials showed that soy protein isolate contained the highest protein (85.08%) content which full fat rice bran was composed of high amount of dietary fiber (18.38%). Both ingredients were aimed to increase nutritional value of corn grits.

The production of direct expanded functional snack food was set to compare between the control formula (CS, normal formula) and the formulas which contained 10.5% soy protein isolate and varied amount of full fat rice bran of 7.5% (FS_1), 10.0% (FS_2) and 12.5% (FS_3). Soy flour was used to reduce vegetable oil content and also to improve homogeneity of fat particles in extrude mixtures. Wheat fiber (VITACEL®) was used to increase dietary fiber content. Calcium carbonate did not

Table 1 Chemical compositions of raw materials.

Raw materials	Content (%)[1]					
	Moisture	Protein[2]	Total fat	Crude fiber	Ash	Dietary fiber
Corn grits	13.08	6.30	0.44	1.67	0.51	3.58
Soy protein isolate	6.07	85.08	3.45	1.41	3.80	8.77
Full fat soy flour	5.57	45.80	23.17	2.12	5.51	20.37
Full fat rice bran	10.45	13.50	16.91	5.49	7.27	18.38

[1] Average of duplicated determination.

[2] N x 6.25

appear in FS_1, FS_2 and FS_3 formula because the results of using it in the lower oil content formula made product surface to crack. The formulas of all ingredients are shown in Table 2.

All four formulas were calculated from Table 1 and Table 2 for the estimation amount of protein and dietary fiber as shown in Table 3.

The results showed that all three formulas contained more protein and dietary fiber as the amount of full fat rice bran content in the formula increased. The% RDI (Recommended Dairy Intake)

of the three formulas for protein was 9.08-10.02% RDI and dietary fiber was 6.85-7.74% RDI which was higher than the control formula.

The comparison of physical properties of all four formulas is shown in Table 4 for expansion ratio, bulk density, hardness and fractal dimension.

The results showed that the soy protein isolate and full fat rice bran which were added to control formula significantly affected the physical properties of the extruded products. The expansion ratio of extrudates decreased significantly ($P \leq 0.05$)

Table 2 Compositions of functional snack food formulas.

Ingredients	Contents (%)[1]			
	CS	FS_1	FS_2	FS_3
Corn grits	93.0	72.5	70.0	67.5
Soy protein isolate	-	10.5	10.5	10.5
Full fat soy flour	-	4.0	4.0	4.0
Full fat rice bran	-	7.5	10.0	12.5
VITACEL®	-	0.5	0.5	0.5
Sugar	3.0	3.0	3.0	3.0
Vitamin-mixed	1.0	1.0	1.0	1.0
Calcium carbonate	1.0	-	-	-
Soybean oil	2.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0

[1] Average of duplicated determination.

Table 3 Total amount of protein and dietary fiber of four formulas[1].

Ingredients	CS		FS_1		FS_2		FS_3	
	Protein (g)	Dietary fiber (g)	Protein (g)	Dietary fiber (g)	Protein (g)	Dietary fiber (g)	Protein (g)	Dietary fiber (g)
Corn grits	5.86	3.33	4.57	2.60	4.41	2.51	4.25	2.42
Soy protein isolate	-	-	8.93	0.92	8.93	0.92	8.93	0.92
Full fat soy flour	-	-	1.83	0.81	1.83	0.81	1.83	0.81
Full fat rice bran	-	-	1.01	1.38	1.35	1.84	1.69	2.30
Total	5.86	3.33	16.34	5.71	16.52	6.08	16.70	6.45
As % RDI	3.52	3.99	9.80	6.85	9.91	7.30	10.02	7.74

[1] Calculated values as wet basis of 100 g.

from 4.20 (CS) to 3.09 (FS_1), 2.88 (FS_2) and 2.68 (FS_3) as the content of the full fat rice bran increased. Bulk density and hardness increased significantly ($P \leq 0.05$) when the soy protein isolate and full fat rice bran added to the formulas compared with control formula (CS), whereas the fractal dimension of all four formulas (1.22-1.24) was not significantly different (P>0.05). It could be stated that the incorporation of soy protein isolate and full fat rice bran into the control formula led to decreasing of product expansion and increasing of hardness. Guy (1994) suggested that during the mixing stage in extrusion process, the proteineceous materials, such as cereal protein and soy protein would absorb water and reduced starch-swelling, causing reducing product expansion. The result showed the decreasing of product expansion and the increasing of harness due to the increasing amount of full fat rice bran in the formulas. Colonna *et al.* (1989) stated that fiber

particles tend to rupture the cell wall in the extrudate, causing reducing in expansion. Guy (1994) reported that at levels of fibrous materials more than 2-3% would affected the expansion and texture of extruded product. Similarly to the work of Onwulata *et al.* (2000) which found that adding cereal fiber more than 10% might cause lower expansion and harder texture of the direct-extruded products.

Chemical compositions of the four formulas functional snack foods are shown in Table 5.

Chemical compositions of the extrudates were determined. Three formulas which composed of 10.5% soy protein isolate and three levels of full fat rice bran of 7.5% (FS_1), 10.0% (FS_2) and 12.5% (FS_3) gained significantly higher contents of protein (18.81-18.87%) than normal formula (7.55%). FS_3, which contained 10.5% soy protein isolate and 12.5% full fat rice bran was composed of the highest dietary fiber contents (6.49%).

Table 4 The physical properties of the products[1].

Formula	Expansion ratio	Bulk density (g/cm^3)	Hardness (Newton)	Fractal dimension (Df)
CS	4.20[a]	0.082[c]	61.49[b]	1.22[a]
FS_1	3.09[b]	0.149[b]	73.48[a]	1.23[a]
FS_2	2.88[c]	0.161[ab]	73.96[a]	1.23[a]
FS_3	2.68[d]	0.185[a]	74.66[a]	1.24[a]

[1] Average of duplicated determination.
[a,b,c,d] Means within the same column with different letters are significantly different   ($P \leq 0.05$).

Table 5 Chemical compositions of the functional snack foods.

Formula	Moisture[1] %	Contents[1] (% as dry basis)				
		Protein	Fat	Crude fiber	Ash	Dietary fiber
CS	4.39[a]	7.55[b]	4.00[c]	0.56[c]	2.43[b]	6.13
FS_1	4.04[ab]	18.87[a]	5.21[b]	0.93[b]	3.21[a]	4.64
FS_2	3.83[ab]	18.87[a]	5.96[ab]	1.53[a]	3.08[ab]	5.95
FS_3	3.45[b]	18.81[a]	6.09[a]	1.74[a]	3.05[ab]	6.49

[1] Average of duplicated determination.
[a,b,c] Means within the same column with different letters are significantly different ($P \leq 0.05$).

Samples from each formula were coated with oil and barbecue seasoning in the appropriate ratio (extrudate : vegetable oil : barbecue seasoning ; 81 : 8.5 : 10.5) and determined for the chemical compositions (Table 6).

Three formulas of barbecue functional snack foods (FS$_1$, FS$_2$ and FS$_3$) contained higher amount of protein, fat, crude fiber and ash than normal formula (CS). The result showed that all 3 formulas, which soy protein isolate and full fat rice bran added contained more than 2 times of protein (16.05-16.12%) than that of the control formula (7.05%). The three formulas contained 2-3 times of protein if compared to the market corn based snack (4.73-8.45%) which reported by Tangkanakul et al., 2000.

Moreover, increasing of full fat rice bran content of 7.5% (FS$_1$), 10.0% (FS$_1$) and 12.5% (FS$_3$) increased dietary fiber contents, especially the FS$_3$ formula, which contained 10.5% soy protein isolate and 12.5% full fat rice bran consisted of the highest dietary fiber contents (5.19%).

The barbecue flavor products were evaluated with the taste panels, using 24 persons and 9-point hedonic scale. Results are shown in Table 7.

The taste panels gave the results that the FS$_1$ and FS$_3$ (the lowest and the highest content of full fat rice bran) were not significantly different for color, odor, flavor and texture. The FS$_2$ showed the highest acceptance by all means with 7.79 score (moderately like) total preference.

From the results of chemical composition and sensory evaluation, the FS$_2$ which contained 10.5% soy protein isolate and 10.0% full fat rice bran was chosen for storage study.

The nutrition values of the barbecue flavor functional snack food (FS$_2$) was determined (Table 8). Iodine was added in the mixture as KI before extrusion step. Vitamin B$_1$ and B$_2$ were filled in the barbecue seasoning before seasoning coated.

The nutritive values of this product calculated

Table 6 Chemical compositions of the barbecue flavor functional snack foods.

Formula	Moisture[1] %	Contents[1] (% as dry basis)				
		Protein	Fat	Crude fiber	Ash	Dietary fiber
CS	3.50[a]	7.05[b]	11.44[b]	0.05[b]	3.90[b]	4.92
FS$_1$	2.66[ab]	16.12[a]	14.13[a]	1.31[a]	4.16[ab]	3.70
FS$_2$	1.97[b]	16.12[a]	14.49[a]	1.15[a]	4.40[a]	4.72
FS$_3$	1.89[b]	16.05[a]	14.51[a]	1.33[a]	4.11[ab]	5.19

[1] Average of duplicated determination.
[a,b] Means within the same column with different letters are significantly different (P ≤ 0.05).

Table 7 Sensory evaluation of the barbecue flavor functional snack foods.

Formula	Score[1]				
	Color	Odor	Flavor	Texture	Total preference
FS$_1$	6.71[b]	6.79[ab]	6.42[b]	6.92[b]	6.96[b]
FS$_2$	7.21[a]	7.04[a]	7.25[a]	7.88[a]	7.79[a]
FS$_3$	6.50[b]	6.29[b]	6.29[b]	6.88[b]	6.42[c]

[1] The scores were 1 = extremely dislike to 9 = extremely like.
[a,b] Means within the same column with different letters are significantly different (P ≤ 0.05).

Table 8   The nutritive values of the barbecue flavor functional snack foods (FS$_2$).

| Compositions	Contents (%)	
	By weight (g/100g)	RDI[1]
Moisture	2.07	-
Protein	15.78	9.46
Fat	14.07	-
Carbohydrate	61.57	-
Crude fiber	2.02	-
Dietary fiber	5.86	7.03
Ash	4.49	-
Vitamin B$_1$	13.93*	278.67
Vitamin B$_2$	10.71*	188.82
Iodine	143.30*	31.40

*   mg/100g
[1]   Recommended Daily Intake for more than 6-year-old Thai people. (Thai RDI)

at 30 g/serving as Recommended Daily Intake (RDI) were 9.46% protein, 7.03% dietary fiber, 278.67% vitamin B$_1$, 188.82% vitamin B$_2$ and 31.4% iodine.

The storage stability was measured by using 2 types of packaging; thin (PET12/MCPP25) and thick (OPP20/PE18/MPET 12/PE 23) metalized polyethylene terephthalate (metalized PET). The products were stored at 35°C and 55°C for 8 weeks. The results are shown in Fig 1-5.

The results showed that the products, which stored at the high temperature (55°C) in thick metalized PET package caused the physical properties, texture and taste-panel acceptance change more than that stored in the thin one. Thin metalized PET was the most suitable package to keep the products within 8 weeks at 35°C, the moisture content (3.72 to 4.87%) and a$_w$ (0.25 to 0.32) slightly increased, the hardness was not significantly different ($P > 0.05$) (84.52 to 90.65
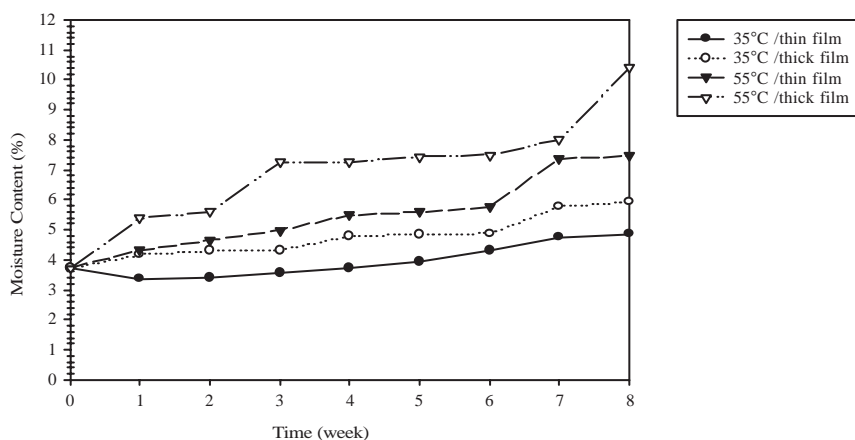
Figure 1   Moisture content of the functional snack food stored in thin and thick metalized PET at 35°C and 55°C.
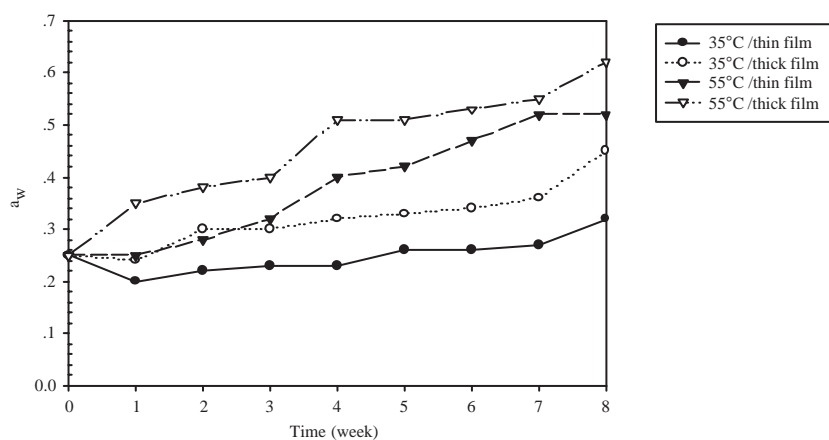
Figure 2 a_w of the functional snack food stored in thin and thick metalized PET at 35°C and 5°C.
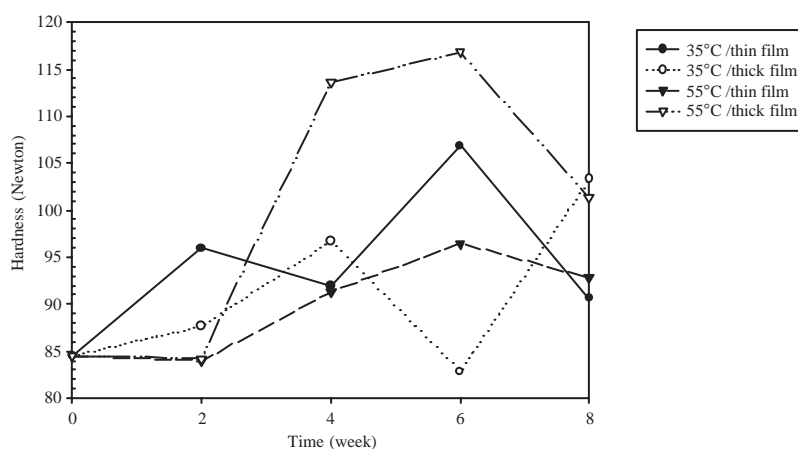
Figure 3 Hardness of the functional snack food stored in thin and thick metalized PET at 35°C and 55°C.

newton), Df slightly decreased (1.24 to 1.13) and the thiobarbituric acid increased (0.20 to 3.24 mg/1000g).

Chemical compositions of 8-week functional snack foods stored in thin and thick package at 35°C and 55°C compared to control sample (no storage) are shown in Table 9.

The functional snack foods were determined by 10-trained panels at weekly interval during a period of 8 weeks against control samples stored in a dark cabinet at -18°C. Sensory scores are shown in Table 10.

The sensory characteristic scores showed

that the functional snack stored in thin metalized PET at 35°C was still accepted by the panels through 8 weeks of storage with 8.4 scores of off-flavor, 9.2 scores of color, 8.0 scores of barbecue flavor and 7.8 scores of crispiness.

The 120-panel with 4 age-groups was asked to give their opinions about appearance, color, odor, flavor, crispiness and total preference for 9-point hedonic scale. Informations are shown in Table 10.

Results indicated that the consumers accepted the functional snack food at the 7-point level of medium-like. People aged 21-30 and 31-40
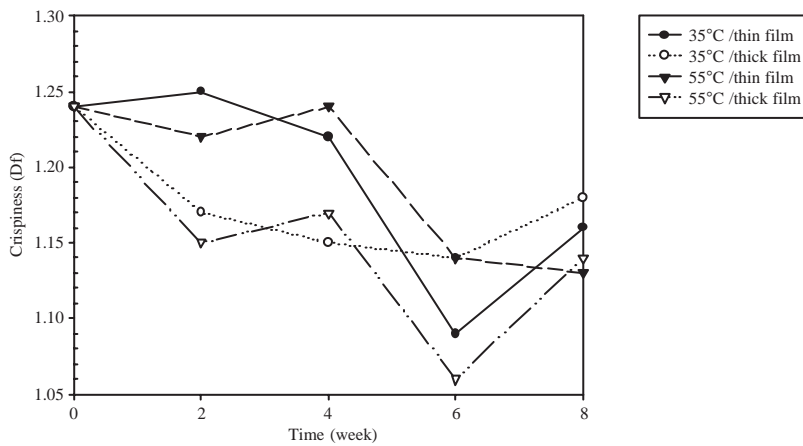
Figure 4 Crispiness (Df) of the functional snack food stored in thin and thick Metalized PET at 35°C and 55°C.
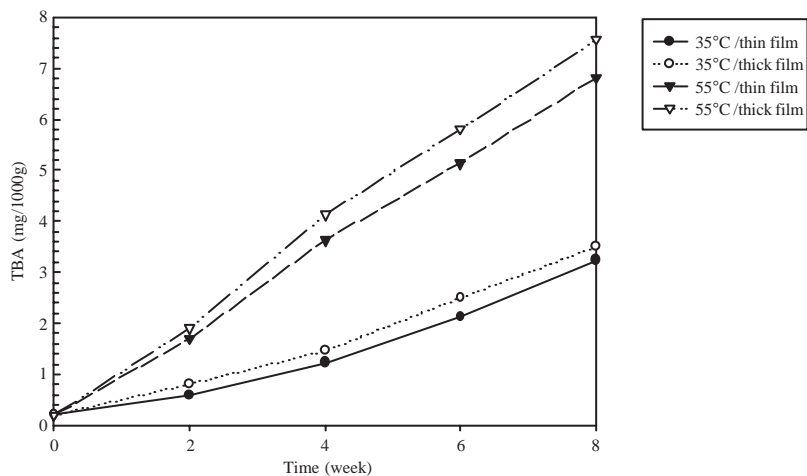
Figure 5 Thiobarbituric acid (TBA) of the functional snack food stored in thin and thick metalized PET at 35°C and 55°C.

years old showed higher acceptance than other age-groups.

CONCLUSION

The acceptable and nutritious functional snack food, which increased protein, dietary fiber, vitamin and mineral was produced by adding 10.5% soy protein isolate and 10.0% full fat rice bran into normal formula.   The product contained 15.78%

protein (9.46% RDI), 5.86% dietary fiber (7.03% RDI), vitamin B_1 13.93 g/100g (278.67% RDI), vitamin B_2 10.71 g/100g (188.82% RDI) and iodine 143.30 g/100g (31.40% RDI). The physical properties and sensory characteristics of the snack depended strongly on the content of soy protein isolate and full fat rice bran in the formulas. The flavor-coated products could store in thin metalized PET at 35°C for at least 8 weeks.

Table 9 Chemical compositions of 8-week functional snack foods stored in thin and thick package at 35°C and 55°C compared to control sample.

Condition		Moisture[1]	Contents [1] (% dry basis)				
Temp.	Package	%	Protein	Fat	Crude fiber	Ash	Dietary fiber
Control		1.97[a]	16.12[a]	14.49[a]	1.15[a]	4.40[b]	4.72
35°C	Thin	4.87[b]	16.06[a]	9.81[b]	1.51[a]	4.11[c]	8.78
	Thick	5.48[c]	15.35[b]	10.30[b]	1.43[a]	4.73[a]	8.25
55°C	Thin	7.59[d]	15.88[a]	11.00[b]	1.42[a]	4.49[b]	8.95
	Thick	10.15[e]	16.09[a]	10.59[b]	1.37[a]	4.73[a]	9.70

[1]　Average of duplicated determination.

Table 10 Sensory characteristics of functional snack foods stored in thin and thick package at 35°C and 55°C for 0-8 weeks compared to control samples.

Characteristic	Temp °C	Package	Time (week)								
			0	1	2	3	4	5	6	7	8
Off-flavor	35	Thin	10.0a	9.8a	9.8a	9.6a	9.4ab	9.4ab	8.8bc	8.6c	8.4c
		Thick	10.0a	9.6ab	9.4b	8.8c	8.8c	8.4cd	8.2cd	8.0d	7.8d
	55	Thin	10.0a	8.8b	7.6c	6.8d	6.6d	5.6e	5.6e	5.6e	5.4e
		Thick	10.0a	8.6b	8.0c	6.6d	6.0e	5.0f	4.4g	4.2g	4.0g
Color	35	Thin	10.0a	10.0a	10.0a	9.8ab	9.8ab	9.8ab	9.6abc	9.4bc	9.2c
		Thick	10.0a	9.8ab	9.8ab	9.6ab	9.4bc	9.0cd	9.0cd	8.8d	8.6d
	55	Thin	10.0a	8.4b	7.8c	7.6c	6.8d	6.8d	6.8d	6.8d	5.2e
		Thick	10.0a	8.8b	7.6c	7.4c	6.2d	5.8d	5.0e	4.4f	4.2f
Barbecue flavor	35	Thin	10.0a	9.8a	9.8a	9.6a	9.4ab	9.4ab	9.0b	8.4c	8.0c
		Thick	10.0a	9.4b	9.2bc	9.0bcd	9.0bcd	8.8cd	8.6d	8.4d	8.4d
	55	Thin	10.0a	8.4b	7.4c	7.2c	7.0cd	6.4d	6.4d	6.4d	5.0e
		Thick	10.0a	8.8b	7.6c	7.4c	6.2d	5.8d	5.0e	4.4f	4.2f
Crispiness	35	Thin	10.0a	10.0a	9.8a	9.8a	9.6a	9.6a	8.8b	8.4b	7.8c
		Thick	10.0a	9.4ab	9.2bc	9.0bcd	8.6cd	8.4d	8.4d	8.4d	7.6e
	55	Thin	10.0a	9.6a	8.6b	7.6c	7.0d	6.6de	6.4e	5.8f	5.0g
		Thick	10.0a	7.8b	6.6c	6.0d	5.8d	5.2e	5.0e	4.0f	4.0f

[a-g]　Means within the same column with different letters are significantly different (P ≤ 0.05).

　　Score　10 = equal to control;
　　　　　　9 = slight difference to control;
　　　　　　8 = more distinct difference but still acceptable;
　　　　　　7 = beginning to lose of acceptability;
　　　　　　6 = more distinct loss of acceptability;
　　　　　　5 = very distinct loss of acceptability;
　　　　　　4 or less = unacceptability.

Table 11 Average sensory scores of functional snack foods gained from 4 age-groups.

Age	Score[1]					
	Appearance	Color	Odor	Flavor	Crispiness	Total preference
10-20 year	5.53[b]	6.27[a]	6.13[b]	6.90[a]	7.37[b]	6.97[a]
21-30 year	6.30[a]	6.43[a]	7.07[a]	6.77[a]	7.63[a]	7.03[a]
31-40 year	6.33[a]	6.23[a]	6.03[b]	6.17[b]	7.17[bc]	7.00[a]
> 41 year	6.37[a]	5.77[a]	6.27[b]	5.90[b]	7.07[c]	6.47[b]

[1]   The scores were 1 = extremely dislike to 9 = extremely like.
[a,b,c] Means within the same column with different letters are significantly different ($P \leq 0.05$).

LITERATURE CITED

A.O.A.C. 1990. Official Methods of Analysis. 15th ed., The Association of Official Analytical Chemists. Arlington, Virginia. 1298 p.

Boonyasirikool, P. and C. Churunuch. 1997. Production of corn–based breakfast cereal by twin screw extruder. Kasetsart J. (Nat. Sci.) 31: 429-444.

Colonna, P., J. Tayeb, and C. Mercier. 1989. Extrusion cooking of starch and starchy products, pp. 247-320. *In* C. Mercier, P. Linko and J.M. Harper (eds.). Extrusion Cooking. American Association of Cereal Chemists, Inc., Minnesota.

Guy, R.C.B. 1994. Raw materials for extrusion cooking processes, pp. 52-72. *In* N.D. Frame (ed.). The Technology of Extrusion Cooking. Blackie Academic and Professional, London.

Kosayothin, A. 1996. Snack Market in 2000: A Perspectives from Co's Food Mogul. Tarnsettakit : 22-24 May, 16 (1996) : 54.

Onwulata, C.I., R.P. Konstance, E.D. Strange, P.W. Smith, and V.H. Holsinger. 2000. High-fiber snacks extruded from triticale and wheat formulations. Cereal Foods World 45 : 470-473.

Reilly, A. and C.M.D. Man. 1994. Potato crisps and savoury snacks, pp. 202-214. *In* C.M.D. Man and A.A. Jones (eds.). Shelf Life Evaluation of Foods. Blackie Academic and Professional, London.

Sinthavalai, S. 1984. Thai Snack Foods : Part I. Basic Information for Product Development. Department of Product Development, Faculty of Agro–Industry, Kasetsart University, Bangkok. 330 p.

Stable Micro System. 1993. Operating Manual Texture Analyzer TA.XT2 Version 5.16. Stable Micro Systems Ltd., England. 79 p.

Tangkanakul, P., P. Tungtrakul and W. Mesomya. 1999. Nutrient contents of commercial snack food products. Kasetsart J. (Nat. Sci.) 33 : 270-276.

Yu, T.C. and R.O. Sinnuhuber. 1957. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. Food Technol. 11 : 104–108.

Effects of Partial Replacement of Rice Flour with Various Starches on the Physicochemical and Sensory Properties of "Sen Lek" Noodle

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and Rasamee Supasri

ABSTRACT

This research was conducted to investigate the effects of partial replacement of rice flour with 5 to 20 % three different starches (potato, corn and cassava) on the chemical and the physical properties of both raw mixed flour and Sen Lek products. Sensory properties of the products were also evaluated. The results indicated that values of protein, amylose, water absorption index (WAI), solid loss as well as the viscosity changes by RVA of the raw mixed rice flour were affected by the level and the types of starch in the mixes. Sen Lek prepared from rice flour containing various starches had higher values of cooking yield and cooking loss than those prepared from pure rice flour (the control). Besides, the products added with starch contained higher carbohydrate but lower protein and fat as the starch content in the raw mixes flour increased. All noodle products, except the one prepared from the mix containing 20% cassava starch, were considerably more elastic than the control. Incorporation of various starches in Sen Lek preparations increased the extensibility and the stickiness values of the cooked products. The sensory evaluation showed that all noodles gave fairly good quality of which three noodle samples replacing with 5% potato starch, 20% corn starch and 10% cassava starch had the highest acceptance scores within each starch group. However, no significant difference was observed on the texture and the acceptance score between those three noodle samples and the control.

Key word: rice noodle, sen lek, rice flour

INTRODUCTION

Rice noodle is one of the products made from rice flour. It is favorably consumed as a main dish or snack by people in south East Asia, Thailand in particular. Through rapid expansion of the noodle's market, locally and internationally, does increase its value. The drawback of the product's qualities still exists due to the instability of the proper quality of raw material, especially rice, and the variation in the production technology. Very few studies have been reported on the desirable rice qualities for rice noodle production (Kohlwey *et al*., 1995; Li and Luh, 1980; Bhattacharya *et al*., 1999). To achieve such quality, a number of rice noodle factories practically added some other native starches or modified starches to replace a portion of rice flour. However, the prevailing of the science data to support the necessary application of those starches to improve the noodle quality is limited. Hence, the physicochemical properties of rice flour mixed with various starches and the qualities of rice noodle were investigated.

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MATERIALS AND METHODS

Raw material

A single lot of rice flour was purchased from a rice noodle factory in the form of rice cake with 40% moisture content. The cake was beaten into a small pieces, dried in a hot-air oven at 45°C for 16 hours and ground with Pin Mill. The flour obtained was kept in the closed plastic bags until the experiment began. Potato starch was obtained from the Winner Group Ltd., while corn starch (Friend ship) and cassava starch (Pha Mung Korn) were purchased from a local supermarket. These three types of native starch are normally used in rice noodle factories.

Flour preparation

Each type of starch was mixed with the rice flour at 5, 10, 15 and 20% of total dry weight. Chemical and physical properties of the flours were analyzed for moisture, protein and ash contents according to AOAC (1990), amylose content by Juliano (1971), water absorption index (WAI) and solid loss by modified Anderson *et al*. (1969). The viscosity of the flours was also measured by using a Rapid Visco Analyser (RVA).

Noodle preparation : Sen lek

Fourty percent of the flour was prepared and let stand for 1 hr at room temperature. Seventy grams of the slurry were poured into 22×28 cm stainless steel tray, steamed for 5 minutes and cooled down to room temperature, resulted in a gelatinized noodle sheet about 1 mm thick .The sheet then was removed from the tray, placed onto the racks and dried on a perforated tray at 150°C for 10 minutes before cooling and cutting to obtain small strips about 0.5 mm wide. The noodles were further dried in a hot-air oven at 45°C until the moisture content decreased to 10-12%.

Physical properties of noodle
Cooking yield and cooking loss

Sen Lek noodles were determined for cooking yield and cooking loss. Noodles of 10 g were cut into 5 cm length and were added to a beaker containing about 150 ml of boiling distilled water on a hot plate. The beaker was covered with a watch-glass, cooked for 10 min and stirred slightly with a glass rod. The cooked noodles were filtered through a stainless steel screen and washed with 20 ml distilled water. After draining for 5 min , the noodles were weighed and the cooking yield was calculated. For cooking loss, the combined filtrate and washing were poured into 200 ml volumetric flask, adjusted to the volume with distilled water. Ten millimeter of the solution was pipetted into a tared aluminum dish ,evaporated until dried and dried at 105°C to constant weight. The solid loss during cooking was calculated.

Tensile strength and stickiness

Noodles were soaked in tap water for 10 min, drained, after 10 min they were cooked in boiling distilled water for 2 min then removed and immediately cooled in distilled water. The cooked noodles were drained on a stainless steel screen for 10 minutes. Texture qualities of the cooked noodles including tensile strength ,break distance (extensibility) and stickiness were measured on a Texture Analyzer model TA-XT2i Stable Micro System Ltd., Vienna ,England., using a Spaghetti Tensile Rig (Code A/SPR) probe and a Pasta Stickiness Rig (Code HDP/PES) probe, respectively.

Chemical properties of noodle

Noodles were determined for moisture, protein, fat and ash contents by the standard methods of AOAC (1990). Carbohydrate content was calculated by the different method 100-(protein + fat + ash content).

Sensory evaluation of noodle

Sensory evaluation of Sen Lek noodles were conducted by using 9 points Hedonic Scale. Twenty

panelists were selected from the Institute of Food Research and Product Development staff. Organoleptic qualities evaluated were appearance, color, shiny and grossy, cohesiveness, flavor, texture and overall acceptance.

Statistical analysis

Data collected from the sensory evaluation were analyzed by the use of ANOVA and mean procedure of SAS (Statistical Analysis System). Duncan's New Multiple Rang Test was used to detect mean differences.

RESULTS AND DISCUSSION

Properties of raw materials

Physical properties such as WAI and solid loss of the mixed rice flour were changed by the levels and the types of starch added in the mixes (Table 1). Rice flour containing 5 to 20% potato, corn and cassava starches had WAI values of 1.29-1.31, 1.25-1.33 and 1.24-1.31%, respectively. As the levels of the cassava starch in the mixes increased, the WAI decreased. Our observation

was in close agreement with the findings of Tiraporn (1990). The cooking losses were relatively high in the samples mixed with corn and cassava starches. The mixes containing potato starch were identical to the pure rice flour (control). Among starch groups, corn starch strongly affected the solid loss of the mixes.

The results from RVA measurement revealed that incorporation of other starches in rice flour affected the rheological behavior on heating and cooling of the flour samples. The mixes with potato and cassava starches gave lower gelatinization temperatures (GT) than the control, while corn starch had no noticeable effect on the GT (Table 2). The mixes with potato starch showed the highest peak viscosity value which implied that this starch can improve swelling and water absorption of the mixes more than the other starches. Final viscosity of the pure rice flour was relatively high, therefore retrogradation easily occurred as compared with the other samples. Among the starch groups, rice flour mixed with cassava starch had the lowest value of the final viscosity resulted in more stickiness of the cooked paste.

Table 1 WAI and solid loss of rice flour and rice flour mixed with various starches.

Sample	WAI*	Solid loss (%)*
Rice flour	1.27	1.63
5% Potato	1.29	1.66
10% Potato	1.30	1.61
15% Potato	1.30	1.65
20% Potato	1.31	1.55
5% Corn	1.33	1.99
10% Corn	1.25	2.02
15% Corn	1.28	2.01
20% Corn	1.28	1.82
5% Cassava	1.31	1.93
10% Cassava	1.28	1.86
15% Cassava	1.29	1.75
20% Cassava	1.24	1.73

* Average are based on three measurements of each sample.

Table 2   Pasting properties of rice flour and rice flour mixed with various starches using Rapid Visco Analyzer (RVA).

Sample	Peak Vis (RVU)	Trough (RVU)	Final Vis (RVU)	Break down (RVU)	Set back (RVU)	Consistency (RVU)	Pasting Temp (°C)
Rice flour	271.08	175.83	359.83	95.25	87.75	184.00	80.75
5% Potato	274.58	137.00	296.58	137.58	22.00	159.58	78.35
10% Potato	298.58	150.08	311.75	148.50	13.17	161.67	77.50
15% Potato	309.67	156.17	312.25	153.50	2.58	156.08	76.70
20% Potato	348.75	178.00	330.58	170.75	-18.17	152.58	73.50
5% Corn	247.50	132.92	280.08	114.58	32.58	147.16	80.75
10% Corn	251.50	138.25	284.92	113.25	33.42	146.67	80.70
15% Corn	237.42	134.92	273.50	102.50	36.08	138.58	80.70
20% Corn	256.62	152.25	301.04	104.37	44.42	148.79	79.15
5% Cassava	256.25	127.00	264.25	129.25	8.00	137.52	79.10
10% Cassava	254.67	127.58	251.00	127.08	-3.67	123.42	78.40
15% Cassava	251.08	124.92	235.50	126.17	-15.58	110.58	76.70
20% Cassava	285.29	155.58	280.33	129.71	-4.98	124.75	75.55

RVU = Rapid Visco Unit

Table 3 showed the chemical composition data of the rice flour and the rice flour mixed with various starches. Increasing amount of starch in the mixes caused a decrease in protein content and an increase in amylose content. Rice flour with 20% corn starch exhibited the highest amylose content (33.40%). However, all samples contain amylose in the range of 30.86 to 33.40% corresponding to the suitable values (27 to 33%) recommended by Niyomvit (1989) for rice noodle manufacturing. There was no change in ash content in all samples.

Noodle qualities

Evaluations of noodle qualities were based on the amount of cooking yield and cooking loss, tensile strength, break distance value, surface stickiness and a taste panel. The results showed that the cooking yield of the noodles with potato and cassava starches were increased as the quantity of the starches in the mixes increased (Figure 1). All noodles prepared from the starches-mixed rice flour

samples had higher cooking yields than the control. This is probably due to the role of the added starches upon the slightly increased water absorption of the noodle. Noodle prepared from the pure rice flour had the cooking loss value of 6.11%. Incorporation of extra starch into rice flour resulted in increasing the solid loss of such noodles during cooking. Sen Lek with 20% corn starch exhibited the lowest cooking loss value (5.09%). Protein and fat contents of the starch added noodle were significantly decreased as the amount of starch increased. Carbohydrate content, on the other hand, were increased in all noodle samples which added starches. Ash content was slightly changed (Table 4).

Textural properties of Sen Lek

It is generally accepted that the main criterion for assessing the overall quality of cooked pasta is based on the evaluation of texture (Smewing, 1997). In this experiment cooked noodles were tested for

Table 3 Chemical compositions of rice flour and rice flour mixed with various starches.

Sample	Protein[a](%)	Ash[a] (%)	Amylose[a](%)
Rice flour	7.41[b]	0.21[b]	30.96[b]
5% Potato	7.15	0.22	30.96
10% Potato	6.56	0.34	30.93
15% Potato	6.38	0.31	31.44
20% Potato	6.20	0.30	31.52
5% Corn	7.09	0.21	31.06
10% Corn	6.77	0.21	32.30
15% Corn	6.40	0.19	32.81
20% Corn	6.18	0.20	33.40
5% Cassava	7.06	0.33	30.86
10% Cassava	6.79	0.31	31.50
15% Cassava	6.26	0.30	31.72
20% Cassava	6.08	0.23	31.83

[a] Values reported on a moisture free basis.
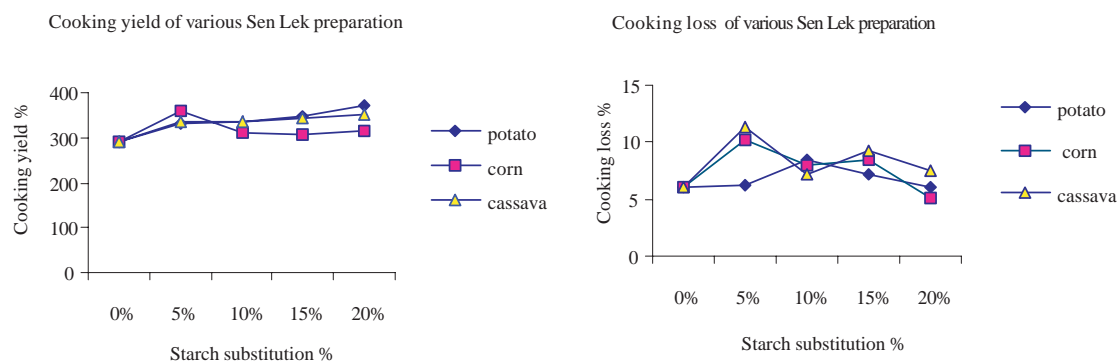[b] Averages are based on three measurements of each sample.

Figure 1 Cooking yield and cooking loss of Sen Lek prepared from rice flour and rice flour mixed with various starches.

tension to assess elasticity and break distance, to imply the extensibility. The results were shown in Table 5. All noodles mixed with starch, except that containing 20% cassava starch, had tensile strength values relatively higher than the control. The noodles with 5% corn, potato and cassava starches had tensile strength values of 45.87, 30.04 and 30.76 gram-force, respectively. While the noodle with 10% potato starch exhibited higher tensile strength value than those with 10% corn starch and 10% cassava starch. However, adding of starch up to 20% resulted in lowering the tensile strength value of the noodle.

It is clear that noodles replacement with potato, corn and cassava starches were considerably more extensible than the control. However, the values were exhibited variations upon the types and the amounts of starch used. Addition of starch also

Table 4 Nutritional value of Sen Lek prepared from rice flour and rice flour mixed with various starches.

Sample	Protein[a] (%)	Ash [a] (%)	Fat [a] (%)	Carbohydrate[a] (%)
Rice flour	7.20	0.24	0.84	91.72[b]
5% Potato	6.95	0.32	0.75	91.98
10% Potato	6.52	0.33	0.82	92.33
15% Potato	5.93	0.31	0.76	93.00
20% Potato	5.51	0.27	0.56	93.66
5% Corn	6.72	0.25	0.71	92.32
10% Corn	6.18	0.23	0.78	92.81
15% Corn	6.22	0.23	0.70	92.85
20% Corn	5.64	0.21	0.66	93.49
5% Cassava	6.56	0.31	0.84	92.29
10% Cassava	6.59	0.29	0.83	92.29
15% Cassava	6.02	0.31	0.80	92.87
20% Cassava	5.67	0.29	0.80	93.24

[a] Values reported on the moisture free basis.
[b] Calculated values, (%) carbohydrate = (100 – Protein-Ash-Fat)

Table 5 Textural characteristics of various Sen Lek from a tensile test cell.

Sample	Max force ± SD*(g)	Distances ± SD*(mm)
Rice flour	29.84 ± 3.36	24.48 ± 2.99
5% Potato	35.34 ± 4.34	29.65 ± 3.09
10% Potato	46.99 ± 6.88	29.05 ±3.14
15% Potato	42.59 ± 6.32	30.76 ± 3.97
20% Potato	33.13 ± 5.85	26.88 ± 2.99
5% Corn	45.87 ± 4.30	34.75 ± 4.11
10% Corn	43.20 ± 6.91	31.06 ± 2.42
15% Corn	40.90 ± 5.56	27.05 ± 2.67
20% Corn	36.84 ± 4.54	26.21 ± 2.93
5% Cassava	30.76 ± 5.03	27.67 ± 3.34
10% Cassava	33.83 ± 2.93	34.60 ± 2.33
15% Cassava	31.24 ± 2.48	35.15 ± 1.51
20% Cassava	26.40 ± 3.44	31.60 ± 3.24

*   Averages are based on 10 measurements of each sample.

affected the stickiness of the cooked noodles as shown in Figure 2. At 20% cassava starch, the noodle possessed the highest value of stickiness, while the lowest value belonged to the sample containing 20%corn starch. The stickiness of the cooked noodles added with cassava starch was increased as the amount of starch increased. The results obtained were similar to that reported by
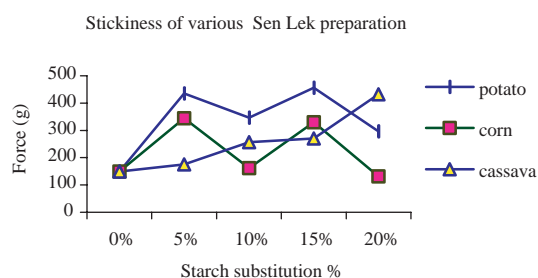
Stickiness of various Sen Lek preparation

Figure 2 Stickiness of Sen Lek prepared from rice flour and rice flour mixed with various starches.

Tiraporn (1990).

Sensory properties

Table 6 showed the results of sensory test of cooked noodles. The noodles with 5 to 20% corn starch and 5- to 20% cassava starch had no significant difference (p ≥ 0.05) based on texture. While the noodle with 10% potato starch had texture score significantly different from the other samples in the group. All noodle characteristics such as appearance, color, shiny, cohesiveness, flavor, texture and acceptability gained preference scores of 6 to7, representing slightly like to moderately like. The noodle samples containing 5% potato starch, 20% corn starch and 10% cassava starch had the highest acceptability scores in each group of the starches. Panelists suggested that the noodle with potato starch gave more elasticity and tougher as the amount of starch increased, while the noodle with cassava starch possessed more extensibility and softer texture than the control noodle. However, at 20% cassava starch, the noodle exhibited undesirable characteristics. In the experiment, texture scores of the noodle prepared from the mixes containing potato, corn and cassava starches significantly correlated with the score of the product acceptability r = 0.952*, 0.926* and 0.941* (p<0.05), respectively. Texture of cooked noodle was the principle characteristics for the panelists to decide the product acceptance (Bhattacharya et al. 1999). In this experiment, it was found that, the panelists could not define any diffrence on texture and overall acceptability between the control and the sample with the highest acceptability score from each group. However, the noodle with 20% corn starch obtained the highest score in both texture and acceptability. This may be caused by the highest amylose content of the mix flour (Sanchez, 1975).

Table 6 Sensory scores of Sen Lek prepared from rice flour and rice flour mixed with various starches.

Sample	Appearance	Color	Shiny	Cohesiveness	Flavor	Texture	Acceptability
5% Potato	6.95[ab]	6.83 [b]	6.57 [ab]	6.48[a]	6.48 [a]	6.76 [a]	6.76 [a]
10% Potato	6.57[b]	6.48[c]	6.24 [b]	6.50 [b]	6.40 [a]	6.14 [b]	6.00 [b]
15% Potato	7.10 [a]	7.10 [ab]	6.33 [b]	6.57 [b]	6.67 [a]	6.86 [a]	6.71 [a]
20% Potato	6.86 [ab]	7.29 [a]	6.86 [a]	6.62 [b]	6.50 [a]	6.60 [ab]	6.33 [ab]
5% Corn	6.15 [b]	6.55 [b]	6.05[c]	6.43 [a]	6.95 [a]	6.43 [a]	6.28 [a]
10% Corn	6.10[ab]	6.70 [ab]	6.35 [ab]	6.75 [a]	6.70 [a]	6.58 [a]	6.58 [a]
15% Corn	6.15 [b]	6.85 [a]	6.20 [bc]	6.75 [a]	6.70 [a]	6.45 [a]	6.45 [a]
20% Corn	7.00 [a]	6.95 [a]	6.50 [a]	6.75 [a]	6.55 [a]	6.75 [a]	6.70 [a]
5% Cassava	6.91 [a]	6.61 [a]	6.59 [a]	6.95 [a]	6.59 [a]	6.41 [a]	6.73 [a]
10% Cassava	6.45[bc]	6.14 [b]	6.18 [bc]	6.55 [ab]	6.27 [b]	6.64 [a]	6.80 [a]
15% Cassava	6.82 [ab]	6.64 [a]	6.50 [ab]	6.36[b]	6.14 [b]	6.59 [a]	6.70 [a]
20% Cassava	6.27[c]	6.59 [a]	6.00 [c]	5.47 [c]	6.00 [b]	6.05 [a]	6.02 [b]

In a column, means followed by the same superscript are not significantly different (P≥0.05) by DMRT

Table 7 Sensory Scores of Sen Lek prepared from rice flour and rice flour mixed with various starches.

Sample	Appearance	Color	Shiny	Cohesiveness	Flavor	Texture	Acceptability
Rice flour	6.45[a]	6.40[bc]	6.52 [b]	6.20 [b]	5.90 [b]	6.40 [a]	6.20 [a]
5% Potato	6.38[b]	6.73 [ab]	6.25 [b]	5.85 [b]	6.25[ab]	6.20 [a]	6.00 [a]
20% Corn	6.90 [a]	7.10 [a]	7.00 [a]	6.88 [a]	6.65 [a]	6.58 [a]	6.65 [a]
10% Cassava	6.08 [b]	6.15[c]	6.18 [b]	6.00 [b]	6.20 [ab]	6.53 [a]	6.03 [a]

In a column, means followed by the same superscript are not significantly different (P≥0.05) by DMRT

CONCLUSIONS

Rice quality had a marked influence on the properties and characteristics of rice noodles. Partial replacement of rice flour with potato, corn or cassava starches at 5 to 20 % by wt affected cooking yield, cooking loss, textural properties and sensory qualities of the noodles.

The products had various characteristics upon the type/ amount of starches added. Potato starch provided noodle with tougher and harder texture as compared with corn starch. Cassava starch, on the other hand, gave noodle with transparent, soft texture and sticky, especially at 20% level. However, all noodles showed fairly good quality, wherein, noodle with 5% potato starch, 20% corn starch and 10% cassava starch had the highest acceptant score in each starch group. No significant difference was observed among those three noodles and the control. Hence, the type/ amount of starches used in this experiment had a minimal affect acceptability of the products. Therefore, the addition of such starches to rice flour in noodle production is not necessary if suitable rice's quality is used as raw material.

ACKNOWLEDGEMENT

LITERATURE CITED

Anderson, R.A., H.F.Conway, V.F. Pfeiffer, and E.L. Griffin Jr. 1969. Gelatinization of corn grits by roll and extrusion cooking. Cereal Sci. Today. 14 : 4-7.

AOAC. 1990. Official Method of Analysis of the Association of Official Analytical Chemists. 15 th ed., Association of Official Analytical Chemists, Arlington, Virginia 1298 p.

Bhattacharya, M., S.Y.Zei, and H. Corke . 1999. Physicochemical properties related on quality of rice noodles. Cereal Chem. 76(6) : 861-867.

Juliano, B.O. 1971. A simplified assay for milled rice amylose. Cereal Sci.Today. 16 : 334-340.

Kohlwey, D.E., J.H. Kendall, and R.B. Mohinda . 1995. Using the physical properties of rice as a guide to formulation. Cereal Foods World 40 : 728-732.

Li, C.F. and B.S. Luh. 1980. Rice snack foods, pp. 670-711. *In* B.S. Luh (ed.) Rice Production and Utilization. Avi Westport, C.T

Niyomvit, N. 1989. Sen Mee. Food Journal. 19(2) : 115-121.(in Thai).

Sanchez, P.C. 1975. Varietal influence on the qualities of Philippines rice. Food Technol. 25 : 51-52 .

Smewing, J. 1997. Analyzing the texture of pasta for quality control. Cereal Foods World 42 : 8-12.

Tiraporn, P. 1990. Effects of mixing tapioca starch and rice flour on rice noodle qualities. M.S. thesis, Kasetsart University. Bangkok.

A Comparative Study on Pretreatment Processes of Canned Whole Kernel Sweet Corn

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ABSTRACT

The effects of pretreatments on yield and qualities of canned whole kernel sweet corn were studied Average yield, drained weight, pH, total soluble solids, and the heating and cooling parameters were determined. The processed canned samples were analyzed for nutrients. It was found that non-blanching process showed more advantages than the process with blanching. The average of yield of the corn kernels, drained weight, total soluble solids, and the nutrient retention of the non-blanching process were higher than the process with blanching, while the weight of the waste residue was lower. However the blanching process has advantage in resulting higher initial temperature (It), shorter rate of heating (fh), rate of cooling (fc) and process lethality at the geometrical center of the can (Fc) from which may result in the shorter thermal process required for the product.

Key words: pretreatment process, canned whole kernel corn

INTRODUCTION

Sweet corn (*Zea mays saccharata L.*) is now becoming an important crop of Thailand. The average production yield per the cultivated area is increasing every year. There are two main markets of the fresh sweet corn, in Thailand, from the field. Seventy percent of the total crop goes to local markets for fresh consumption, while the other 30% is used as the raw material in canning industries. The important exported corn products are canned whole kernel sweet corn and frozen sweet corn. In 1997, the exported volume of sweet corn products was 19, 283 tons with the value 488.9 million baht. In 1996, Thailand was the sixth canned sweet corn exporting country of the world and had a share in the market at 3.6% of the world volume. However, the expanded volumes of Thai sweet corn products during 1991-1996 were about 7.8-13.4% annually. In 1998, there were 16 sweet corn canning factories in 11 provinces in Thailand, from which built up the demand for fresh sweet corn of 185,251 tons/year. Factories in Kancha-naburi were the major group which used sweet corn up to about 80,000 tons/year or about 43.2% of the country's total demand for fresh sweet corn as raw material (Suriyo *et al.*, 1999).

Since canned sweet corn is one of the important canned vegetables, it is included in the proposed Draft Codex Standard for Certain Canned Vegetable 2002 which was prepared by France and Thailand. In the draft standard of step 3, scope, description, essential composition and quality factors, food additives, contaminants, hygiene, labelling, weight and measures, and analysis and sampling methods of the products were established

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(FAO/WHO/CODEX 2002). Nowaday most consumers are interested in reading the nutrition labelling of canned foods. From randomization, about 58% of consumers read nutrition labelling on canned foods before deciding to buy (Shine *et al.*, 1997). Although , there are no present mandatory nutrition labelling requirements for food in the Southeast Asian region, except for special catogories of foods and when nutrition claims are made. There is, however, increasing interest among authorities in the region in formulating regulations for nutrition labelling for a wider variety of foods (E-Siong-Lee, 2,000). The consumer requirement for nutrition labelling may increase more later on, depending on consumers' education and well being. In order to upgrade the canned products' quality, improvement of the canning process is necessary.

Nowaday, there are two different pretreatment processes in canning of whole kernel sweet corn in Thailand. These processes are no blanching and blanching of whole kernal sweet corn before canning (Figure 1). Although each pretreatment process is set to suit the available machines in the factories, the advantages and disadvantages of the processes are not certainly known.

Thus, the purpose of this study is to find out the advantages and disadvantages of the pretreatments, in order that canning processors can decide to manage their canning line of canned whole kernal sweet corn for better quality canned products.

MATERIALS AND METHODS

1. Materials

1.1 Fresh sweet corns (Insee 1) from the National Corn and Sorghum Research Center, Kasetsart University, Pakchong, Nakhon Ratchasima, Thailand.

1.2 Tap water for preparing the packing medium, blanching and cooling.

1.3 Lacquer coated metal cans, number 2

A (307 × 409).

1.4 Four different brands of commercial canned whole kernel corns were sampled from the west, central, north-east and north of Thailand for microbiological and nutrients analysis.

2. Methods

2.1 Fresh corns were husked, divided and separately followed the processes of canned whole kernel sweet corn as indicated in Figure 1. The blanching time was 5 minutes, filled weight of corn was 350 gm/can and packing medium was boiling water. The filled cans were 5 minutes exhausted in a steam exhaust box and the thermal process was 20 minutes at 121.1°C in a small vertical retort (Taylor).
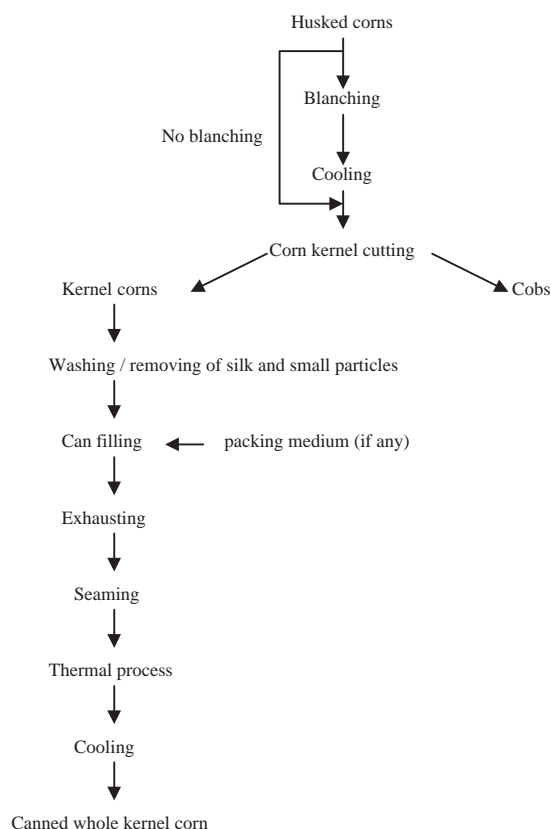
2.2 Samples were also heated with

Figure 1 The process chart of canned whole kernel sweet corn with blanching and no blanching.

thermocouples inserted at the geometrical center of the cans for heat penetration studies. These samples were also exhausted, lid closed and processed at 121.1°C Can temperatures were recorded every minute until the cooling process finished. The data obtained were used to plot heating and cooling curves on 3-cycle logarithmic papers. The rate of heating (f_h) and cooling (f_c), initial temperature (IT) and the process lethality at the geometrical center (F_c) of the cans were obtained.

2.3 The canned samples from 2.1 and the commercial samples were examined for the presence of microbiology by the use of total plate count, and for mesophilic aerobic sporefor-mers, thermophilic flat sour sporeformers, mesophilic anaerobes, thermophilic anaerobes and sulfide spoilage (Kautter *et al.*, 1992)

2.4 For physical examination, 2-week stored canned samples were determined for net weight and drained weight (drained on a sieve with the openings 2.8 mm × 2.8 mm. for 5 mins). The canned net content was blended in an electric blender for 3 mins., and determined for pH (Orion pH-meter) and brix (Atago hand refractometer). Enough samples were sent to IQA-Norwest Labs for analysis of nutrients, in percentages. The analysis methods were as follows:-

Calories and Calories from fat (by Calculation)
Total Fat (AOAC 2002, 922.06)
Saturated Fat (AOAC 2000, 963.22)
Cholesterol (J AOAC, 1993)
Sodium (AOAC (2000), 968.08)
Total Carbohydrate (by Calculation)
Dietary Fiber (AOAC (2000), 985.29)
Total Sugar (JAOAC, 1992)
Protein (N×6.25) (AOAC (2000), 981.10, and Tecator Application Note)
Vitamin A (In-house method based on Liquid Chromatography and Analysis of Food and Beverages, Vol.2)
Vitamin C (JAOAC, 1992)
Calcium (AOAC (2000), 968.08)
Iron (AOAC (2000), 968.08)
Ash (AOAC (2000), 942.05)
Moisture (AOAC (2000), 950.46 B)

2.5 The canning experiments were done in duplicate. The data obtained was statistically analysed for significantly differences at $p<0.05$ by Analysis of Variance Program.

RESULTS AND DISCUSSION

From Table 1, we can compare the yields of the whole kernel corn from the different pretreatment processes. The blanching prior cutting process showed a little less in yield than the unblanching process, this might be due to the loss of some soluble solids during / after blanching the corns However, there was no significant difference ($p<0.05$). The weight of cobs passed blanching was heavier than that of non-pretreated process. This might be due to the water-absorbing of the substances, mostly celluloses, of the cobs during blanching and cooling. However, there was no significant difference ($p<0.05$). The heavier weight of the waste due to water absorbing rendered further

Table 1 The weight of the whole kernels and cobs after the different pretreatments (based on the weight of fresh raw material).

Treatment	% weight	
	Whole kernel	Cob
No blanching	60.20[a]	39.80[a]
Blanching	59.92[a]	45.79[a]

In a column, means with the same letter are not significantly different ($p<0.05$).

problem in the waste disposal with faster rate of decomposition, and consequently induced undesirable smell.

　　　Table 2 showed the average drained weight, pH and °B of the canned samples. With the equal filling weight, the blanched cans showed smaller drained weight than the non-blanched one. This might be due to heating process which changed some insoluble substances such as pectic substance, starch, cellulose, etc., to soluble substances and streamed out to the surrounding liquid. The blanched sample passed more heat treatment and cooling so that more weight loss and total soluble solids (as °B) occured. Normally pH of the foodstuffs is lower than 7, due to the acidity of the food constituents. Loss of some food constituents, especially volatile acids, during blanching will raise the pH of the food toward neutral (Meyer, 1960)

　　　Table 3 showed the thermal process parameters. The average initial temperature of the blanched canned samples were significantly higer than the unblanched one. This might be due to the heat from blanching was leftover. Even the blanched corn was cooled down, it may be difficult for the heat to transfer from the gelatinized starch in the inner part of the kernel. However, this is the advantage of the blanching treatment, because it caused the rate of heating (fh) and the process lethality (Fc) faster than the unblanching process. Blanching also has another advantage in reducing the number of microorganisms contaminated with the raw material, resulting in lower process-time needed than the unblanching process. After heating, most of constituents have changed, thus during cooling, the rate of cooling (fc) was slower than fh. The rate of cooling of the blanched treatment from which contained lower drained weight and lower soluble solids was significantly shorter than fc of

Table 2 The average of drained weight, pH and °B of the two pretreated canned whole kernel sweet corn samples.

Treatment	Average drained weight, %	Average pH	Average total soluble solids (°Brix)
No blanching	63.34	6.56	6.8
Blanching	62.74	6.91	6.0

Table 3 Some thermal process parameters of canned whole kernel sweet corn samples.

Treatment	Average IT,°C	RT, °C	Come-up time,(min)	Average fh (min)	Average fc (min)	Average Fc (min)
No blanching	84.6[a]	121.1	5	5.3[a]	22.3[a]	7.80[a]
Blanching	86.3[b]	121.1	5	4.5[a]	16.0[b]	7.25[a]

In a column, means with the same letter are not significantly different(p<0.05).

Note　IT = initial temperature of the canned samples

　　　RT = retort temperature

　　　Come-up time = the time required to reach retort temperature after the steam is turned on

　　　fh = heating rate of the canned samples at the geometric center

　　　fc = Cooling rate of the canned samples at the geometric center

　　　Fc = Process lethality at the geometrical center of the cans

the unblanched treatment.

The microbiological examination of the canned samples which had processed for 20 mins. at 121.1°C was shown in Table 4. There was negative test on the microbial growth. All the commercial samples had also negative test.

Table 5 showed the range of nutrient content of the commercial canned samples and the average nutrient content of the prepared canned samples. There were factors affecting the difference in nutrients of the commercial samples, such as the corn's varieties, maturity, location of plantation, post harvest treatment, process pretreatment, strength of packing medium (which may be brine, syrup or water) etc. However, with the comparison between the non-blanched and the blanched prepared

Table 4 Microbiological examination of canned whole kernel sweet corn samples.

	Commercial samples	No blanching	Blanching
Total plate count (CFU/g)	none	none	none
Mesophilic aerobic sporeformers	negative	negative	negative
Thermophillc flat sour sporeformers	negative	negative	negative
Mesophilic anaerobes	negative	negative	negative
Thermophilic anaerobes	negative	negative	negative
Sulfide spoilage	negative	negative	negative

Table 5 The average nutrient content of the commercial canned samples and the prepared samples.

Nutrient	Commercial samples per 100 g	No blanching per 100 g	Blanching per 100 g
Calories, cals	77.1-95.8	74.23	74.25
Calories from fat, cals	10.26-20.2	16.38	14.33
Total fat, g	1.14-2.25	1.82	1.60
Saturated, fat, g	0.12-0.54	0.32	0.26
Cholesterol, mg	0	0	0
Sodium, mg	117-252	1.12	1.26
Total carbohydrate, g	14.36-17.99	11.67	12.21
Dietary fiber, g	2.96-4.12	3.11	3.15
Sugars, g	4.33-7.81	3.13	2.91
Protein (N×6.25), g	2.35-2.83	2.79	2.76
Vitamin A, IU	ND	ND	ND
Vitamin C, mg	0.49-2.60	2.36	1.38
Calcium, mg	1.80-2.61	3.89	7.63
Iron, mg	0.17-0.33	0.29	0.27
Ash, g	0.57-0.98	0.39	0.36
Moisture, g	77-81.3	83.4	68.1

ND = Not detected at a lower limit of detection

samples, the non blanched samples showed higher nutrient content than the blanched samples, except in the content of dietary fiber, sodium and calcium.

CONCLUSION

Non blanching process of canned whole kernel sweet corn showed more advantages than the process with blanching. The yield in weight of the corn kernels, drained weight, total soluble solids, and the nutrient retention of the non-blanching process were higher than the blanching process. Moreover the weight of cobs which was the waste residue was less in the non blanching process, thus providing ease in the waste treatment.

However the blanching process has advantage in the higher initial temperature, shorter rate of heating and cooling, and process lethality from which may result in the shorter thermal process required for the product.

ACKNOWLEDGEMENT

LITERATURE CITED

AOAC, 2000. Official Methods of Analysis. Vol.. 2. 17th ed. AOAC International Suite 500, Maryland, USA. 58 p.

E-Siong-Lee 2000. Labelling, nutrition and health claims : What's happening in Southeast Asia ? Food-Australia 52 (6) : 249-251.

JAOAC, 1992. Journal of AOAC International. The Scientific Association Dedicated to Analytical Excellence. May / June 1992, Vol. 75, No 3, Arlington, Va., USA. 590 p.

JAOAC, 1993. Journal of AOAC International. The Scientific Association Dedicated to Analytical Excellence. July / August 1993, Vol. 76, No 4, Arlington, Va., USA. 943 p.

Kautter, D.A., W.L. Landry, A.H Schwab, and G.A. Lancette, 1992. Examination of canned foods, pp. 259-280. *In* Food and Drug Administration, Bacteriological Analytical Manual, 7th edition. AOAC International, Arlington, VA.

Meyer, L.H. 1960 Food Chemistry. Reinhold Publishing Corporation, New York. Charles E. Tuttle Company, Tokyo. 379 p.

Shine, A, O'. S. Reilly, and O'. K. Sullivan, 1997 Consumer attitudes to nutrition labelling. British Food Journal 99(88) : 283-289.

Suriyo, S., J. Boonmaak, and N. Wuthiwan, 1999 The extension of sweet corn production of Dept. of Agricultural Extension. Presented in The 6th Seminar of Corn Industry Aug. 4-6, 1999. Pakchong Landmark Hotel, Pakchong, Nakhon Ratchasima, Thailand (in Thai) 13 p.

The Proposed Draft Codex Standard for Certain Canned Vegetable, step 3 (2002). FAO/WHO (CODEX) 19 p.

Nutritional Evaluations of Green Catfish, *Mystus nemurus*

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ABSTRACT

The nutritive values of green catfish, *Mystus nemurus* such as proximate analysis, fatty acid profile (especially omega – 3 fatty acids, EPA : eicosapentaenoic acid; DHA : docosahexaenoic acid), amino acid contents, vitamins and minerals were studied. The composition of dried green catfish and sardine meal were also carried out. The results showed that percentages of protein, fat, moisture in fresh, dried green catfish, *Mystus nemurus* and sardine meal were 18.43, 65.99, 68.80; 4.93, 22.40, 7.78 and 75.75, 7.04, 6.17, respectively. Vitamin E content in dried green catfish (264 µg/100 g) was higher than that in sardine meal (84 µg/100 g) while calcium and phosphorus in dried green catfish were lower than those in sardine meal. Fatty acid contents especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in dried green catfish (0.4256, 1.7472 g/100 g) were also found to be higher than those in sardine meal (0.0467, 0.1011 g/100 g respectively). The results of this study indicated that dried green catfish, *Mystus nemurus* were high in nutritive values especially omega – 3 fatty acids (EPA, DHA). The green catfish, an inland fish, was also found to have higher omega – 3 fatty acids than marine fish, sardine.

Key words: green catfish, *Mystus nemurus*, eicosapentaenoic acid, docosahexaenoic acid, omega – 3 fatty acid

INTRODUCTION

Green catfish, *Mystus nemurus* (Cuv. & Val.) a common name of this catfish (Suvatti, 1950), is a fish in Siluroidei family. It has 4 pairs of barbels which were nasal barbels, maxillary barbels, mandibulary barbels and mental barbels but has no scale. Fishes in gastrointestinal tract of green catfish are many kinds such as *Cyclocheilichthys apogon, Puntius tetrazona, P. fasciatus, Rasbora* sp. etc. Green catfish finds the food at night time (NamPong Env. Mgt. Research Project, 1980).

Mystus nemurus has been found from Indochina and Thailand to Malaya and Java, attaining a length in some cases of nearly 60 cm, although individuals of 25-35 cm are more common. It occurs throughout the lengths of many rivers, from the headwaters down to the mouths, where they may be found in brackish water. There seems to be no evident preference for either clear or muddy environments. They dine on a variety of items, among which are crustaceans (crabs, prawns), aquatic and terrestrial insects, fishes, and vegetation. Among the fishes identified as eaten were species of Clarias and Kryptopterus. One specimen was reported as having its stomach crammed full of large red ants. Females from 12.3 to 32 cm long

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contained enlarged ova, the 32 cm specimen with eggs that measured 1 mm in diameter. This species has a thin black lateral stripe at all sizes and a black spot at the end of the adipose fin (Burgess, 1989; Sriwatana and Kasisuwan, 1996).

The spawning season is not sharply defined and is protracted. A fish 32 cm long taken in the Chantabun River at Chantabun June 11, 1926, had very large ovaries with nearly ripe eggs 1 mm in diameter, while fish in spawning condition have been observed in the Menam Chao Phya in November (Smith, 1945).

Production of green catfish from many fishery sources is not constant because green catfish fishery lacks of academic document of feeding method and green catfish strain (Amatyakul *et al.*, 1995). Nowadays it is known that omega-3 polyunsaturated fatty acids (n-3 PUFAs) intake relates with low risk incidence of coronary heart disease especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) n-3 PUFAs. EPA and DHA can decrease metabolism of eicosanoid in blood platelet which may inhibit incidence of atherosclerosis and hypertension (Kinsella *et al.*, 1990). These omega-3 are found from both marine and inland fish.

Many studies showed that omega-3 fatty acids were very important to life development because docosahexaenoic acid (DHA) was found in brain and retina. It was believed that brain and visual development of infant relates with DHA. So it was recommended that infant milk should be supplemented with omega-3 fatty acids. In according to the importance of omega-3 fatty acid for life, the experts from many countries define that omega-3 fatty acid must be enough consumed. Public Health Ministry of England defines dietary reference value of omega-3 fatty acid not less than 0.2 percent of energy intake (Dahlan, 1995).

The objective of this research was to demonstrate the nutritional values of green catfish especially omega-3 fatty acid such as eicosapentaenoic acid (EPA) and docosahexaenoic

acid (DHA) to provide more complete informations in feeding and intake of green catfish. Another objective was to compare the nutritional values especially omega-3 fatty acid between green catfish which is inland fish and sardine meal which is marine fish.

MATERIALS AND METHODS

Sample preparation

The edible portions including skin of eight months old green catfishes, with an initial weight of 500–550 g were obtained from Songkhla Inland Fisheries Station, Department of Fisheries, Ministry of Agriculture and Cooperatives to Institute of Food Research and Product Development, Kasetsart University, Bangkok. They were fed with fishes composed of 72.72 % moisture, 21.10 % protein, 0.28 % fat and 3.14 % ash. Fresh green catfishes were dried in hot air oven at 40°C for 6 hours and continued drying at 60°C for 15 hours. Then dried green catfish was blended into powder. From 4700 g fresh green catfish was dried and blended into 1285 g dried green catfish so the percentage of the yield was 27. After that fresh green catfish, dried green catfish and sardine meal were determined for nutritional evaluation.

Analytical procedures

Three kinds of fish were determined for proximate analysis by AOAC method (1998). Cholesterol and free fatty acid were analysed by gas chromatography (AOAC 1998). Mineral were analysed by atomic absorption (AOAC 1998). Vitamins were analysed by HPLC and microbioassay method. Amino acid composition was determined by a high speed amino acid analyzer (Hitachi Model L-8500, Japan) and tryptophane was analysed (Matheson, 1974).

RESULTS AND DISCUSSION

Table 1 showes the highest percentage of

moisture in fresh green catfish (75.75) whereas the lowest percentage of moisture in sardine meal (6.17). It showed the highest percentage of protein in sardine meal (68.80) which was nearly the same as in dried green catfish (65.99). The percentage of fat in dried green catfish was higher (22.40) (nearly 3 times) than in sardine meal (7.78). Khan *et al.* (1993) reported that the percentage of moisture, protein, fat and ash in green catfish fed 27 % protein and 10 % fat were 75.50, 14.90, 5.85 and 3.75 respectively. It showed that moisture and fat content in fresh green catfish in this experiment were the same as the study of Khan *et al.*, in spite of difference of fat content feeding. In this experiment, fresh green catfish was fed with 0.28 % fat whereas in Khan *et al.*'s experiment, fresh green catfish was fed with 10.00 % fat. Protein content (18.43 %) of green catfish in this experiment was higher than in

Khan *et al.*'s experiment (14.90 %). However protein content (21.10 %) of feeding diet in this experiment was lower than in Khan *et al.*'s experiment (27 %).

Table 2 shows that mineral contents of sardine meal were higher than fresh and dried green catfish. This may be because the sardine meal was prepared from the whole fish whereas the green catfish was prepared only from the edible portion. The sardine meal in this experiment showed higher calcium, phosphorus, iron, copper and zinc than the sardine produced from roller dried fish which head and bone were cut (Phithakpol *et al.*, 1984).

Table 3 shows that vitamin C was found only in fresh green (460 µg/100 g). Vitamin B_1, B_6, E, folic acid and pantothenic acid in dried green catfish were higher than in fresh green catfish and sardine meal. The sardine meal in this experiment

Table 1 Proximate analysis of green catfish and sardine meal.

Fish	Moisture	Protein	Fat	Ash
		(g / 100 g)		
Fresh green catfish	75.75	18.43	4.93	0.59
Dried green catfish	7.04	65.99	22.40	3.74
Sardine meal	6.17	68.80	7.78	15.62

Table 2 Mineral contents of three kinds of fish.

Mineral	Fresh green catfish	Dried green catfish	Sardine meal
		(mg / 100 g)	
Calcium	18.17	257.68	4660.95
Phosphorus	165.72	685.90	2938.65
Sodium	60.82	165.47	545.36
Potassium	216.09	817.42	940.08
Magnesium	28.19	85.70	211.16
Iron	1.00	4.58	35.93
Iodine	0.03	0.09	0.21
Copper	0.07	0.22	1.24
Zinc	0.99	3.43	9.13
Chloride	36.43	124.71	447.56

Table 3 Vitamin contents of three kinds of fish.

Vitamin	Fresh green catfish	Dry green catfish (μg / 100 g)	Powder sardine
Vitamin C	460	0	0
Vitamin B_1	20	90	20
Vitamin B_2	50	140	240
Vitamin B_6	140	450	80
Vitamin B_{12}	2	6	8
Vitamin E	20	264	84
Folic acid	5	38	22
Niacin	2800	12000	13800
Pantothenic acid	1810	5590	240
Biotin	1	4	28

had similar vitamin B_1 content (20 μg / 100 g) to in the report of Phithakpol *et al*. (1984).

Table 4   shows that valine was the first limiting amino acid in fresh green catfish and sardine meal. Amino acid contents in dried green catfish were more complete than those in fresh green catfish and sardine meal. All amino acid contents of sardine meal were lower than roller dried sardine (Phithakpol *et al*., 1984).

The result from table 5 shows that free fatty acid contents of fresh green catfish were the lowest whereas all free fatty acid contents of dried green catfish were the highest except lauric acid which was lower than in sardine meal. The interesting free fatty acids were eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of dried green catfish which were 9 and 17 times higher than sardine meal respectively inspite that green catfish is inland fish

Table 4 Essential amino acid composition of three kinds of fish.

Amino acid	Fresh green catfish	Dried green catfish (mg / g protein)	Sardine meal	FAO / WHO[2]
Tryptophane	16	11	12	10
Threonine	49	47	41	40
Isoleucine	43	44	37 (92)[1]	40
Leucine	84	84	72	70
Lysine	99	97	77	55
Methionine + Cystine	42	41	36	35
Phenylalanine +				
Tyrosine	78	79	115	60
Valine	46 (92)[1]	51	44 (88)[1]	50

1   Limiting amino acid with chemical score
2   Source : Food Composition Table for Use in East Asia (FAO, 1972)

$$\text{Chemical score} = \frac{\text{amino acid content in fish}}{\text{amino acid content in FAO / WHO standard}} \times 100$$

Table 5 Fatty acid composition of three kinds of fish.

Fatty acid	Fresh green catfish	Dried green catfish (g / 100 g)	Sardine meal
Lauric	0.0049	0.0448	0.0467
Myristic	0.1232	0.6944	0.1867
Palmitic	1.2276	5.3312	3.2987
Palmitoleic	0.1775	1.0752	0.0545
Stearic	0.5670	2.6208	0.6146
Oleic	1.5579	6.9888	2.7463
Linoleic	0.5571	2.3296	0.5835
Linolenic	0.0246	0.2688	0.0156
Octadecatetraenoic	0.0000	0.0000	0.0000
Arachidic	0.0394	0.4032	0.0233
Gadoleic	0.0789	0.0000	0.0000
Eicosapentaenoic	0.0887	0.4256	0.0467
Behenic	0.0197	0.0672	0.0233
Erucic	0.1183	0.4256	0.0311
Docosahexaenoic	0.3352	1.7472	0.1011

whereas sardine is marine fish. The reason may be dried green catfish was composed of 22.40 % fat content whereas sardine meal was composed of 7.78 % fat content. But fresh green catfish was composed of 4.93 % fat content which was lower than fat content (7.78 %) of sardine meal. Nevertheless EPA and DHA in fresh green catfish were 2 and 3 times higher than in sardine meal respectively. Cholesterol contents of fresh green catfish, dried green catfish and sardine meal were 42.50, 268.30 and 63.60 mg / 100 g respectively.

CONCLUSION

Eight months-old green catfish fed with 21.10 % protein, 0.28 % fat, with weight of 500 – 550 g from Songkhla Inland Fisheries Station were determined for nutritional value compared with sardine meal. The results showed that the percentage of protein, fat and moisture of fresh green catfish, dried green catfish and sardine meal were 18.43, 65.99, 68.80; 4.93, 22.40, 7.78 and 75.75, 7.04,

6.17 respectively. Mineral contents of green catfish were lower than sardine meal. Vitamin C was found only in fresh green catfish. Vitamin E content of dried green catfish (264 µg / 100 g) was higher than sardine meal (84 µg / 100 g). Valine was the first limiting amino acid in fresh green catfish and sardine meal. Amino acid contents in dried green catfish were more complete than those in sardine meal. The results of fatty acid contents of green catfish were very interesting because two omega-3 fatty acids, which eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of dried green catfish were higher than sardine meal. It may be because of higher fat and cholesterol contents of dried green catfish than sardine meal. Nevertheless fresh green catfish showed lower fat and cholesterol contents than sardine meal but the interesting results showed that omega – 3 fatty acid contents, EPA and DHA were 2 and 3 times higher than sardine meal respectively. Eventhough green catfish is inland fish whereas sardine is marine fish. Since omega-3 fatty acid (especially EPA and DHA) were found

in brain and retina, food containing high EPA and DHA may reduce the risk of hyperlipidemia, high blood pressure and coronary heart disease. So green catfish consumption may be very useful for health of normal population and patients from those diseases except diabetes mellitus.

LITERATURE CITED

Amatyakul C., M. Manote, W. Sriwatana, S. Sumanochitraporn, P. Sripatrprasite, S. Chesoh, A. Sihirunwong, S. Sihirunwong, S. Kasisuwan, and W. Leelaviwat. 1995 . Green Catfish. Inland Fisheries Division, Departmentof Fisheries, Ministry of Agriculture and Cooperatives. 56 p.

Association of Official Analytical Chemists. 1998. Cholesterol in foods, pp 73-75. *In* Official Methods of Analysis of AOAC International. 16th ed. Gaithersburg, Maryland.

Burgess W.E. 1989. An Atlas of Freshwater and Marine Catfishes, A Preliminary Survey of the Siluriformes. T.F.H. Publication, Inc. United States. 784 p.

Dahlan V. 1995. Omega-3 polyunsaturated fatty acids and their new roles in medicine and industry, pp 20-23. *In* FoSTAT Annual Report 1995-1996. Food Science and Technology Association of Thailand.

Food and Agricultural Organization of the United Nations. 1972. Food Composition Table for Use in East Asia. U.S. Department of Health, Education and Welfare. U.S. Government Printing Office. 334 p.

Khan M.S., K.J. Ang, M.A. Ambak, and C.R. Saad. 1993. Optimum dietary protein requirement of a Malaysian freshwater catfish, *Mystus nemurus*. Aquaculture 112 (2/3) : 227 – 235.

Kinsella J.E., B. Lokesh, and R.A. Stone. 1990. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease : possible mechanisms. Am. J. Clin. Nutr. 52 : 1-28.

Matheson N.A. 1974. The determination of tryptophane in purified proteins and in feeding – stuffs. Br. J. Nutr. 31 : 393-399.

NamPong Env. Mgt. Research Project. 1980. Water weeds and studies on fish, fish production and productivity. Inter Committee for Coordination of Investigationson the Lower Mekong Basin, Working Document No. 12. Edited and published by the Mekong Secretariat. 56 p.

Phithakpol B., C. Wongkhalaung, C. Piemsomboon, L. Loohaviranit, L. Herborg, P. Jittanoonta, P. Yamali, S. Chanyavilas, and V. Na Thalang. 1984. Phase III : Marketability and feasibility of pilot plant production of roller dried fish in Thailand. The Food and Agricultural Organization of the United Nations. 74 p.

Smith H.M. 1945. The Fresh- Water Fishes of Siam or Thailand. United States Government Printing Office, Washington. 622 p.

Sriwatana W. and S. Kasisuwan. 1996. Effect of stocking densities on growth and yield of the green catfish, *Mystus nemurus* (Cuv. and Val.) cultured in floating cages. Technical Paper. No. 30/1996. Inland Fisheries Division, Department of Fisheries, Ministry of Agriculture and Cooperatives. 37 p.

Suvatti C. 1950. Fish of Thailand. Royal Institute, Thailand. 379 p.

Stable Carbon and Nitrogen Isotope Ratios of Sediment in Ban Don Bay: Evidence for Understanding Sources of Organic Matters in the Coastal Environment

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ABSTRACT

Sedimental cores from Ban Don Bay and adjacent areas were collected by gravity core sampler for examination of $\delta^{13}C$, $\delta^{15}N$, organic carbon and nitrogen contents. The $\delta^{13}C$ of surface (0-1 cm) sediments from the whole sampling area ranged between –28‰ and -20‰. The $\delta^{15}N$ values of sediments near the river mouth were somewhat higher than those in the outer part of the bay. Sediments with high organic carbon content occurred in the river and the most outer part of the bay. Organic nitrogen contents in the sediment showed almost same pattern as those of organic carbon contents. The atomic ratios of carbon to nitrogen were high in the river and river mouth. These ratios decreased with the increase in distance from the river mouth. Overall, our results clearly demonstrated most of the terrestrial organic matters discharged from the river into the Ban Don Bay had been deposited onto the bottom sediment inside the bay, they had not been expanded cover to the Angthong Islands.

Key words: Ban Don Bay, stable carbon isotope, stable nitrogen isotope, sediment, coastal environment

INTRODUCTION

Ban Don Bay is one of the productive coastal areas in the southern part of Thailand. This bay is located in Surat Thani Province, Southern Thailand. The bay recieves surface freshwater runoff from rivers and canals such as Tapi River, Thathong Canal, Donsak Canal. The fishery productions in the bay have now disappointingly been decreased. This decrease was probably caused by over fishing and environmental change. It is said that the increasing population and the intensification of agro-industrial activities gradually have caused serious aquatic environmental problems in this area.

The Ban Don Bay has frequently been contaminated with direct and indirect discharges of untreated industrial and domestic wastes passing through canals and rivers from the town. Intensive shrimp farming has rapidly expanded to coastal areas around the bay during the last two decades. Many mangrove forests were accordingly cut down for shrimp pond digging. Since the average feed conversion ratio of shrimp was estimated to be about 2.0-2.5, large amounts of organic matter were daily input into the ponds (Musig *et al*., 1995). Effluents from shrimp farmings also caused pollution problems in aquatic environments nearby. This bay has thus faced with problems of organic matter accumulating from the land by recent man-

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made activities and natural runoff of river. However, the extent of the effects of these materials on the whole bay is poorly known.

Sediment research is one useful approach for the study of these questions. One way to evaluate the extent to which sedimentary records reflect actual biological and geographic sources is to directly compare the compositions of organic materials settling through the water column with those preserved in the underlying sediments. This present study is the first attempt to use stable carbon and nitrogen isotope techniques to understand the coastal environment of the Ban Don Bay. The stable carbon and nitrogen isotopes have been used by a number of authors to identify the origins of organic matters (Thornton and McManus, 1994; Guo *et al.*, 1996; Meksumpun *et al.*, 1998a) and to determine the trophic structure of marine communities (Wada *et al.*, 1991; Parson and Chen, 1995; Wu *et al.*, 1997). Mishima *et al.* (1996) have tried to estimate the movement of both terrestrial and marine organic matters in the Osaka Bay (Japan) by using the stable carbon and nitrogen isotopes technique.

Here we report a study of the carbon and nitrogen contents, and the stable carbon and nitrogen isotope ratios of particulate organic materials in surface sediments of the Ban Don Bay. Other related aquatic factors have also be integrated. The goal of this study is to understand the terrestrial organic material movement and coastal environment of the Ban Don Bay.

MATERIALS AND METHODS

At least two sedimental cores were sampled from each sampling station in the Ban Don Bay and adjacent area during August 18-20, 1999. The map of the sampling locations (12 stations) was shown in Figure 1. Water depth and salinity were also measured with STD meter (Mini STD SD202). The water depth and surface salinity contour lines were shown in Figures 2 and 3, respectively. The sediment

cores were sectioned for every 1 cm interval and immediately frozen at -40 $^\circ$C until further analysis.

The sediment samples were freeze-dried in laboratory. The dried sediment samples were then ground and packed in silver cups. In order to remove carbonate, the sediments in silver cups were treated with 1N-HCl solution. They were again dried and packed in tin cups prior to analysis. The total organic carbon and nitrogen contents, together with carbon and nitrogen isotopic compositions in sediment samples were obtained using a continuous flow analytical system joining an elemental analyzer (Carlo Erba, NA-1500) with a stable isotope ratio analyzer (Finnigan MAT252). Measuring standards consisted of CO_2 gas produced from NBS18 standard and N_2 gas produced from L-alanine. The final results are reported as $\delta^{13}C$ and $\delta^{15}N$ (‰) relative to the Peedee belemnite (PDB) limestone standard (carbon) and atmospheric N_2 (nitrogen), as defined by the following equation:

$$\delta^{13}C \text{ or } \delta^{15}N \text{ (‰)} = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 100$$

where $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$. Data quality control throughout the analysis was ensured by running a reference standard after every 10 runs. The analytical precision for standard preparation and mass spectrometric analysis was less than ± 0.1‰ and ± 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

RESULTS

Stable carbon isotope ratio ($\delta^{13}C$)

The $\delta^{13}C$ value of surface sediments in the river at station 1, where salinity was estimated to be 0 psu, was –27.8‰. The highest $\delta^{13}C$ value of surface sediment obtained at station 10 with the value of –20.5‰. The contour map of $\delta^{13}C$ values of surface sediments (0-1 cm) in the Ban Don Bay is shown in Figure 4. The $\delta^{13}C$ values of the surface sediments increased gradually with increasing distance from the river mouth. However, the vertical profiles of $\delta^{13}C$ values at most sampling stations

were generally constant. Table 1 shows the vertical profiles of $\delta^{13}C$ values in sediments at stations 1, 6 and 11.

Stable nitrogen isotope ratio ($\delta^{15}N$)

Figure 5 showed the contour map of $\delta^{15}N$ values of surface sediments in the Ban Don Bay. The $\delta^{15}N$ values of the surface sediments near the river mouth were generally higher than those around the Angthong Islands (Figure 5). The lowest $\delta^{15}N$ value was found at station 12 (3.8‰).

Organic carbon and nitrogen contents

Total organic carbon contents of the surface sediments in the Ban Don Bay and adjacent area were ranged between 6.20 and 14.75 mg/g (dry weight). The highest organic carbon content was found at station 12 at value of 14.74 mg/g. Low organic carbon contents (lower than 10.00 mg/g) were found at the river mouth. The contour map of organic carbon content of the surface sediment in the bay and adjacent area was shown in Figure 6.

The highest nitrogen content of the sediment was found in station 12, closed to Samui and Phangan Island, at the value higher than 2.00 mg/g. Figure

7 showed contour map of organic nitrogen content of sediment in the study area. The organic nitrogen content showed almost same pattern as that of the organic carbon content. Low nitrogen contents of the sediments were found in the stations near the river mouth.

Atomic C:N values of the sediments were shown in Figure 8. The values were usually high (ranged from 11.7 to 13.9) in the sediment obtained from river and closed to the river mouth (stations 1-3). The lowest C:N value was found at station 12. The C:N ratios in the other areas (stations 4-8) varied between 9.5 and 10.3 with an average of 9.72 (SD ± 0.63). The C:N ratios of the surface sediments at station 9-11 varied between 8.3 and 8.4 with an average of 8.3 (SD ± 0.06).

DISCUSSION

Data analysis for $\delta^{13}C$

In general, the value of $\delta^{13}C$ of terrestrial organic matter and marine organic matter are distinctly different. Stable carbon and nitrogen isotope compositions of organic matter in sediment had been studied to examine the movement of

Table 1 Vertical profiles of stable carbon isotope ratios ($\delta^{13}C$) of sediment samples collected in August 1999.

Station 1		Station 6		Station 11	
Sediment depth (cm)	$\delta^{13}C$ (‰)	Sediment depth (cm)	$\delta^{13}C$ (‰)	Sediment depth (cm)	$\delta^{13}C$ (‰)
0-1	-27.7	0-1	-21.9	0-1	-20.7
1-2	-27.6	1-2	-21.8	1-2	-20.5
2-3	-27.6	2-3	-22.2	2-3	-20.5
3-4	-27.6	3-4	-21.7	3-4	-20.6
4-5	-27.8	4-5	-21.9	4-5	-20.7
5-6	-27.6	5-6	-21.8	5-6	-21.0
6-7	-27.7	6-7	-21.3		
		7-8	-21.3		
		8-9	-21.7		
		9-10	-21.3		

terrestrial organic matter by several workers (e.g. Thornton and McManus, 1994; Yamada *et al.*, 1996; Mishima *et al.*, 1996; Meksumpun *et al.*, 1998b). Although the values of $\delta^{13}C$ and $\delta^{15}N$ of particulate organic matter often showed distinct seasonal variations (e.g. Voss *et al.*, 1996), the particulate matter in water column and sedimental samples collected from off shore areas in the same time for $\delta^{13}C$ and $\delta^{15}N$ determinations were reported to have no statistical difference at the α=0.10 level and were assumed to be isotopically equivalent (Goering *et al.*, 1990). In this study, the levels of $\delta^{13}C$ ranged from –27.8‰ at the riverine station to more than –20.4‰ in the area from station 10. Such $\delta^{13}C$ data thus indicated no effect of major terrigeneous materials in sediments of the area from station 10 to the outer part of the bay. Here the $\delta^{13}C$ value of terrestrial organic matter and marine organic matter were approximately –27.5‰ and –20.5‰, respectively. The $\delta^{13}C$ data of this study corresponded with the report of Gearing *et al.* (1984) in which the $\delta^{13}C$ of phytoplankton collected from Malaysian water was -21.0‰. Moreover, the data were also well corresponded with several authors who found that the $\delta^{13}C$ for coastal and offshore marine sediments ranged from -23‰ to -18‰ (e.g.Tan *et al.*, 1991; Meksumpun *et al.*, 1998b), whereas the $\delta^{13}C$ for terrestrial organic matters ranged from -28‰ to -26‰ (e.g. Tan *et al.*, 1991; Thornton and McManus, 1994; Mishima *et al.*, 1996). Watanabe *et al.* (1997) had also shown that the mean $\delta^{13}C$ value of sediment and suspended solids in the Choa Praya estuary at the upper most sampling station was close to -26.5‰.

Since the $\delta^{13}C$ of the marine planktonic source was definitely different from those of most terrestrial organic material sources, we could clarify the impact of terrestrial organic matter on the marine ecosystem. In order to estimate the movement pattern of terrestrial organic matter which had been loaded from the river into the Ban Don Bay, the percentages of terrestrial organic carbon (T) in each sampling station were calculated by the following equation:

$$T\ (\%)\ =\ \frac{\delta^{13}C_{\ marine} - \delta^{13}C_{\ sample}}{\delta^{13}C_{\ marine} - \delta^{13}C_{\ terrestrial}}\ \times\ 100$$

$\delta^{13}C_{\ marine}$: $\delta^{13}C$ of marine organic matter
$\delta^{13}C_{\ sample}$: $\delta^{13}C$ of measured sample
$\delta^{13}C_{\ terrestrial}$: $\delta^{13}C$ of terrestrial organic matter

Based on our $\delta^{13}C$ data, we decided the end members of $\delta^{13}C_{\ marine}$ and $\delta^{13}C_{\ terrestrial}$ to be –20.5‰ and –27.5‰, respectively. Our calculated data showed that the percentages of terrestrial organic matters in the surface sediment decreased gradually from about 70 % in the area near the river mouth to less than 20 % in the outer area close to the Angthong Islands (Figure 9). Such occurrences were considered to be due to comparative weak current of water movement so as the loaded particles can be easily sinked down onto the bottom deposits.

Data analysis for $\delta^{15}N$

The $\delta^{15}N$ values of the sediments of sampling stations in the Ban Don Bay (stations 1-6) were markedly higher than those of stations nearby Angthong Islands (stations 10-12). Base on these results, together with the results of $\delta^{13}C$ analyses, the data suggested that organic matters in those two areas, which may contribute substantial inputs of carbon and nitrogen onto the sediments, had different isotopic compositions. As previously reported by Goering *et al.* (1990) that the $\delta^{15}N$ values of mixed phytoplankton dominated by diatom (*Thalassiosira aestivalis*, *Skeletonema costatum* and *Chaetoceros debilis*) collected in Auke Bay during the prebloom in spring was 3.3 ± 0.6‰, the results here which indicated the $\delta^{15}N$ values of 3.8 ± 0.1‰ in sediment collected at station 10-12 may imply the dominance of diatoms in the water column along those stations. Although there were some differences in origin of organic deposition, the ranges of $\delta^{15}N$ in our results could clearly confirm that the sediments in the outer area were mostly derived from the primary production in overlying water column.
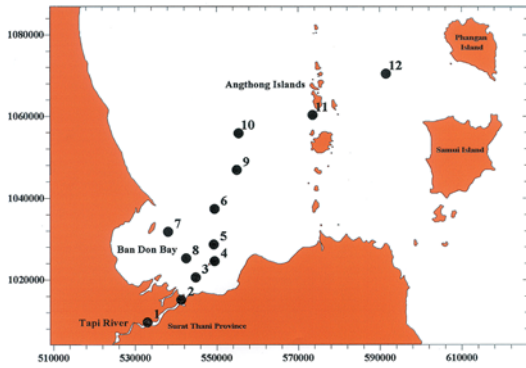
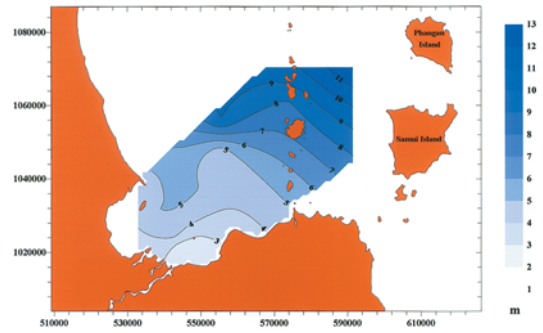**Figure 1** Sampling stations in the Ban Don Bay and adjacent area in Surat Thani province, Thailand.

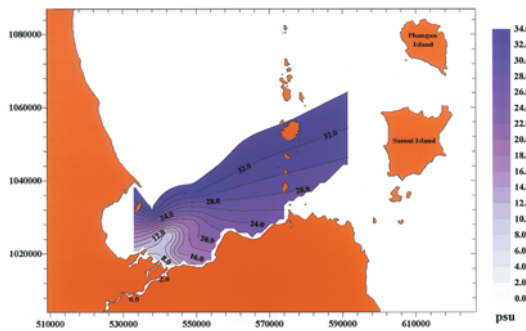**Figure 2** Contour graph of water depths in the Ban Don Bay and adjacent area.

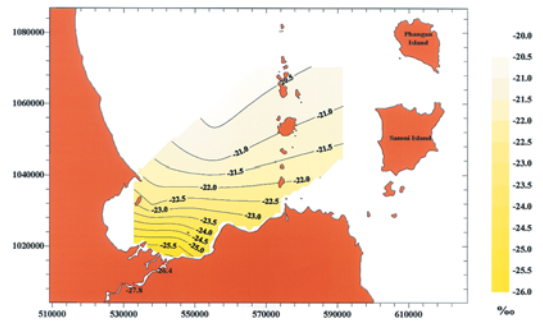**Figure 3** Contour graph of surface salinities (psu) in the Ban Don Bay and adjacent area.

Figure 4 Contour graph of carbon isotope ratios (δ^{13}C) of the surface (0-1 cm) sediments in the Ban Don Bay and adjacent area.
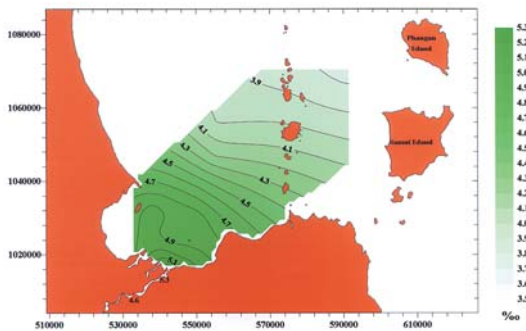

Figure 5 Contour graph of nitrogen isotope ratios (δ^{15}N) of the surface (0-1 cm) sediments in the Ban Don Bay and adjacent area.
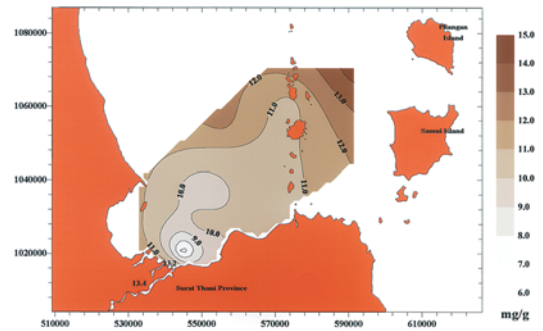

Figure 6 Contour graph of total organic carbon contents of the surface (0-1 cm) sediments in the Ban Don Bay and adjacent area.
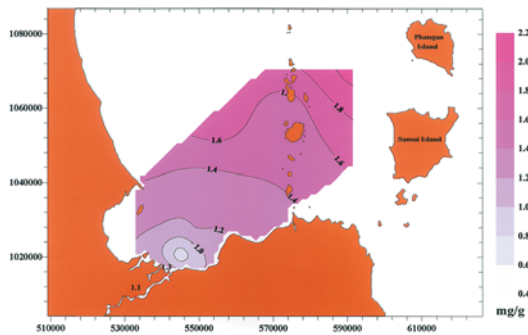
Figure 7 Contour graph of total organic nitrogen contents of the surface (0-1 cm) sediments in the Ban Don Bay and adjacent area.
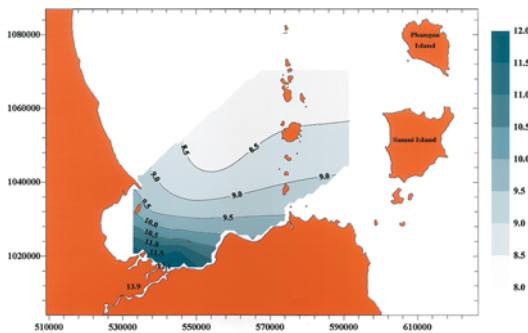

Figure 8 Contour graph of C:N ratios of the surface (0-1 cm) sediments in the Ban Don Bay and adjacent area.
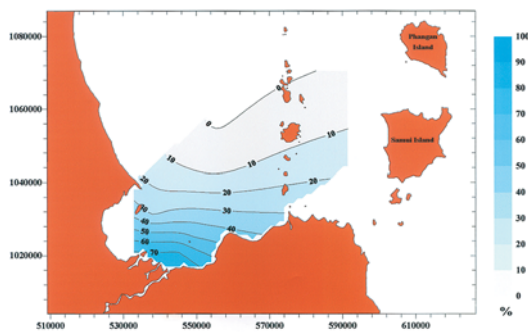

Figure 9 Contour graph of percentages of terrestrial organic matters in surface (0-1 cm) sediments in the Ban Don Bay and adjacent area.

Data analysis for organic carbon and nitrogen contents

An accumulation patterns of organic carbon and nitrogen should depend upon the distribution of water masses and currents which were in turn influenced by wind velocity and direction and topography. Because of the surface current velocity and direction in the Ban Don Bay were remarkably influenced by the monsoon, the accumulation patterns of organic carbon and nitrogen were considered to be directly controlled by monsoon-induced currents in the whole study area. Distributions of organic contents of carbon and nitrogen of surface sediment showed almost the same pattern. Although low carbon and nitrogen contents of surface sediment were found in small areas closed to the river mouth, the mean values of the organic carbon and nitrogen contents of the whole sampling area were still high.   The organic contents of carbon and nitrogen of surface sediments in some parts of the study area were as high as the high production areas e.g. in the Osaka Bay (12-22 mg/g for carbon and 1.8-2.4 mg/g for nitrogen), Japan (Montani *et al*., 1991; Mishima *et al*., 1996). Such high concentrations of organic materials deposited in the Ban Don Bay may be one of the reasons that causes this area characterizes as a comparatively high fishery production zone of the Gulf of Thailand.

Data analysis for C:N ratios

Atomic C:N ratios of particulate organic matter have been employed as source indicators of sedimentary particulate organic matter by numerous workers (e.g Prahl *et al*., 1980; Thornton and McManus, 1994).   Additionally, Hedges *et al*. (1988) indicated that the elemental compositions from trap samples in various water depths were similar to those of the underlying surface sediments, and the C:N ratio of the particulate organic matters in the upper layer of water column was only slightly lower than those in the lower layers. Our C:N distribution clearly showed that the C:N ratios of

surface sediment close to the river mouth were higher than those in the Ban Don Bay and these ratios gradually decreased from the river to the outer part of the bay. Generally, the high values of C:N ratios (>10) of sediments from mid-latitude areas had been interpreted to be a large effect of terrigenous materials input (Thornton and McManus, 1994; Mishima *et al.*, 1996). The results from $\delta^{13}C$ clearly demonstrated that the sediments close to the river mouth were affected by terrigeneous materials, whereas those in stations nearby Angthong Islands were mostly derived from authchthonous primary production. The mean value of C:N ratio of area that marine organic sources should be the major contributor to the sediment organic matter pools (stations 9-12) was estimated to be 8.3 ± 0.2. Tan *et al.* (1991) have similarly revealed that the C:N ratios of sediment in the East China Sea, which contain a dominant contribution of marine organic carbon, lie in the range 6.2 to 8.8.

In conclusion, the distribution pattern of stable isotope composition of sediments in Ban Don Bay implied that most of terrestrial organic matter discharged from the river into the Ban Don Bay had entirely been deposited onto the bottom sediment within the Ban Don Bay. Such discharges could not reach the Angthong Islands.

LITERATURE CITED

Gearing, J.N., P. J. Gearing, D.T. Rudnick, A.G. Requejo, and M.J. Hutchins. 1984. Isotopic variability of organic carbon in a phytoplankton-based, temperate estuary. Geochemica et Cosmochimica Acta. 48 : 1089-1098.

Goering, J., V. Alexander, and N. Haubenstock. 1990. Seasonal variability of stable carbon an nitrogen isotope ratios of organisms in a North Pacific Bay. Estuarine, Coastal and Shelf Science. 30 : 239-260.

Guo, L., P.H. Santschi, L.A. Cifuentes, S.E. Trumbore, and J. Southon. 1996. Cycling of high-molecular-weight dissolved organic matter in the carbon isotopic (^{13}C and ^{14}C) signatures. Limnol. Oceanogr. 41 : 1242-1252.

Hedges, J.I., W.A. Clark, and G.L. Cowie. 1988. Organic matter sources to the water column and surficial sediments of a marine bay. Limnol. Oceanogr. 33 : 1116-1136.

Meksumpun, S., C. Meksumpun, A. Hoshika, T. Tanimoto and Y. Mishima. 1998a. The use of stable isotopes for understanding coastal environment of the Hiroshima Bay. Scientific Report of Chugoku National Research Institute. 51 : 17-24.

Meksumpun, S., C. Meksumpun, A. Hoshika, Y. Mishima, and T. Tanimoto. 1998b. Stable isotope technique for evaluation of organic matter movement and coastal environment status., pp.189-204. *In* D. Almoza and H.M. Ramos (eds.). Applied Sciences and Environment. WITpress. Southampton.

Mishima, Y., A. Hoshika, and T. Tanimoto. 1996. Movement of terrestrial organic matter in the Osaka Bay, Japan, pp. VI-20-25. *In* Proceedings of the third International Symposium of ETERNET-APR : conservation of the Hydrospheric Environment, 3-4 December 1996, , Bangkok.

Montani, S., Y. Mishima, and T. Okaichi. 1991. Scavenging processes of marine particles in Osaka Bay. Marine Pollution Bulletin EMECS'90. 23 : 107-111.

Musig, Y., W. Ruttanagosrigit, and S. Sampawapol. 1995. Effluents from intensive culture ponds

of Tiger Prawn (*Penaeus mododon* Fabricus). Kasetsart University Fishery Research Bulletin. 21 : 17-24.

Parsons, T.R. and Y.-L L. Chen. 1995. The comparative ecology of a subarctic and tropical estuarine ecosystem as measures with carbon and nitrogen isotopes. Estuarine, Coastal and Shelf Science. 41 : 215-224.

Prahl, F.G., J.T. Bennett and R. Carpenter. 1980. The early diagenesis aliphatic hydrocarbons and organic matter in sedimentary particulates from Dabob Bay, Washington. Geochemica et Cosmochimica Acta. 44 : 1967-1976.

Tan, F.C., D.L. Cai, and J.M. Edmond. 1991. Carbon isotope Geochemistry of the Changjiang Estuary. Estuarine, Coastal and Shelf Science. 32 : 395-403.

Thornton, S.F. and J. McManus. 1994. Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: Evidence from the Tay Estuary, Scotland. Estuarine, Coastal and Shelf Science 38 : 219-233.

Voss, M., M.A. Altabet, and B.V. Bodungen. 1996. δ^{15}N in sedimenting particles as indicator of euphotic-zone processes. Deep-Sea Research. 43 : 33-47.

Wada, E., H. Misutani, and M. Minagawa. 1991. The use of stable isotopes in food web analysis. Critical Reviews in Food Science and Nutrition 30 : 361-371.

Watanabe, H., K. Ohta, K. Sawada, and Y. Nozaki. 1997. Distribution of terrestrial organic matter in the Choa Praya estuary (Thailand). An abstract presented in the Seminar on Geochemistry (Chikyu-Kagaku) 17-19 September 1997, Tokyo, Japan. (in Japanese)

Wu, J., S.E. Calvert, and C.S. Wong. 1997. Nitrogen isotope variations in the subarctic northeast Pacific: relationships to nitrate utilization and trophic structure. Deep-Sea Research. 44 : 287-314.

Yamada, Y., T. Ueda, and E. Wada. 1996. Distribution of carbon and nitrogen isotope ratios in Yodo River watershed. Jpn. J. Limnol. 57 : 467-477.

Potential Biodiesel Production from Palm Oil for Thailand

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ABSTRACT

The purpose of this study is to review and evaluate aspects on Thailand's energy situation, oil palm plantation, properties of palm oil, conversion process to biodiesel, suitably available lands, biodiesel quality, environmental impacts, engine test performance and benefits of the country from using biodiesel. The results show that if 20% of diesel production from the amount of imported crude oil in 2000, was compensated by this biodiesel production, it would reduce imported crude as estimated value as Bt 13,436 million. To avoid conflict with feedstock for food production, plantation areas of at least 4.4 million rais in 12.9 million rais (1 rai = 0.16 ha) of suitably available lands will be required. Oil Palm is quantitatively the highest commercially potential production among the existing Thailand's major oil crops. Transesterification can provide chemical transformation of crude palm oil (CPO) and crude palm kernel oil (CPK) to biodiesel. The product of this process has been acceptable as diesel fuel substitute. Environmental impacts from biodiesel utilization show positive results compared to diesel fuel No. 2 (DF2). Biodiesel is acceptable as alternative diesel fuel with no significant problems found in both direct and indirect injections. Conclusively, the results of this review and evaluation show high potential biodiesel production from palm oil for Thailand.

Key words: biodiesel, palm oil, conversion, environmental impacts, engine tests

INTRODUCTION

There is no argument that energy is one of the essential things for our everyday living. In fact, most of today utilized energy derived from fossil resources, which are non-renewable energy. Notably, world reserves of oil, natural gas, coal and uranium are being depleted and predicted length of their supply as no longer than 40-50, 55-60, 150 and 80 years, at today world consumption rate, respectively (El Bassam, 1998; Connemann and Fischer, 1999; Crabbe *et al*., 2001). It is then feasible that the prices of petroleum and natural gas are likely to increase or fluctuate regarding the resource reduction.

In Thailand, the consumption of crude and refined oil, in the year 2000, were around 92 Ml/day (50.57% of total commercially energy consumption); whereas, local crude oil production could supply only 9.2 Ml/day (10% of total consumption of crude and refined oil). Nevertheless, imported crude oil was around 102 Ml/day worth Bt 285,862 million per annum as 86% of total commercial primary energy import. Within the final modern energy consumption produced from crude oil, diesel fuel consumption was around 41 Ml/day, which was 49% of total petroleum products (NEPO, 2001).

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Technically, 23.5% of refinery input (crude oil) is basically conversed to DF2 (diesel fuel for transportation) as an output (Sheehan *et al*., 1998). Therefore, to calculate the money saving from reducing imported crude oil procurement for diesel production, the equivalent value of diesel within imported crude (without refinery cost) is around Bt 67,178 million per annum. Supposably, if the country were able to save this part of imported crude oil around 20% (4.79 Ml/day), it would be equivalent to at least Bt 13,436 million per annum. Meanwhile, Thailand, with high capability in agricultural production or biomass, is able to provide the local substitute primary energy for diesel fuel, this amount of money will therefore circulate within the country.

Bioenergy, the sustainable resource

As stated earlier, to prolong or maintain not only as the renewable energy sources and production but also being environmental friendly to the world and local habitats, the term "sustainable" is therefore represented. Many researches confirmed that the bioenergy or biofuel, as a Biomass (as products of plant and animal matters on the earth's surface), is able to address many of the key issues and problems surrounding sustainable development, including combating global climate change, supporting and creating jobs, strengthening rural economics, enhancing the rural environment and recycling resources (Chamber, 2000).

In the work of El Bassam (1998), the comparison among fossil, nuclear and biomass as the energy sources considering on social, economic and environmental aspects of utilization were analyzed. The results have shown that biomass had taken on benefits over the others, especially on renewable, CO_2 reduction, landscape, accident risks, costs of environmental repair, administrative costs, creating new jobs, decentralization of economic structure, improving farmer's incomes, significant time of waste decay, public opinion, genetic deformation, etc. (Table 1)

Biodiesel, the study purpose

The National Energy Policy Committee, NEPC (2001), regulated biodiesel as the fuel for diesel engine produced from vegetable oil, which is transformed into methyl or ethyl ester. The vegetable oil for biodiesel manufactured typically contains up to 14 different types of fatty acids (Tyson, 2001). Popularly, the term "biodiesel" is now usually referring to esters of vegetable oils or animal fats or waste oils and not the corresponding feedstocks. DF2 is the fuel with which biodiesel is usually compared (Knothe and Dunn, 2001). The primary purpose of this study is to review and evaluate on country's energy situation, oil palm plantation, properties of palm oil, conversion processes, suitably available lands, biodisel quality, environmental impacts, engine test performance and country's benefit from using biodiesel.

MATERIALS AND METHODS

In this study, a model of biodiesel production from palm oil is presented as shown in Figure 1, which is fundamentally based on the literature review from Cook (1984); Berger (1984); Kulavanich *et al*. (1988); Gervasio (1996); Van Dyne *et al*. (1996); Muniyappa *et al*. (1996); El Bassam (1998); Noureddini *et al*. (1998); Sheehan *et al*. (1998); Canakci and Van Gerpen (1999); Connemann and Fischer (1999); Ma and Hanna (1999); Demirbas (2000); Crabbe *et al*. (2001); IEM (2001) and Knothe and Dunn (2001).

Oil palm

Oil palm has its scientific name as Elaeis guineensis Jacq. The genus Elaeis is derived from the Greek word "elaion" meaning oil. The specific name guineensis indicates its origin in the Guinea Coast (Salunkhe *et al*., 1992). Oil palm was brought to plant in Thailand before the World War II; consequently, the first commercial plantation started at Krabi and Satun Provinces since 1968. Currently, the popular commercial oil palm species, planted in

Table 1 Social, economic and environmental aspects of utilization of different energy sources (El Bassam, 1998).

	Fossil	Energy fuels Nuclear	Biomass
Renewable	no	no	yes
CO_2 reduction	no	yes	yes
Reduction of heat emission	no	no	yes
Landscape	no	no	yes
Avoidance of large accident risks	no	no	yes
Excessive costs of environment repair	yes	yes	no
Reduction of administrative costs	no	no	yes
Innovation	no	yes	yes
Creating new industrial jobs	no	yes	yes
Promoting decentralization of economic structure	no	no	yes
Promoting export	no	yes	yes
Increasing autonomous energy supply (industrialized countries)	no	yes	yes
Increasing autonomous energy supply (developing countries)	no	no	yes
Improving farmers' incomes	no	no	yes
Significant time of waste decay	yes	yes	no
Migration to urban areas	yes	yes	no
Favorable public opinion	no	no	yes
Avoidance of international conflict and wars	no	no	yes
Genetic deformation	no	yes	no
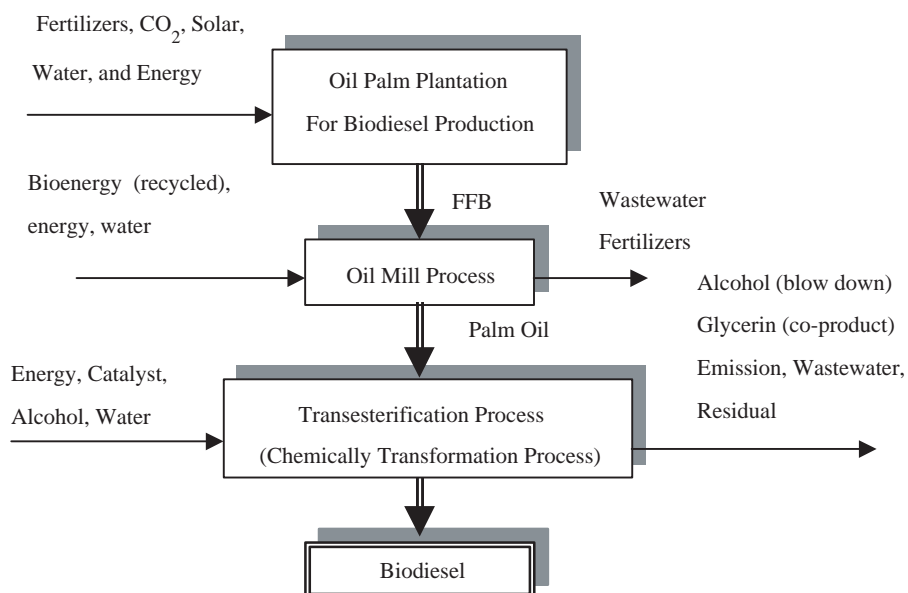
Figure 1 A model overall process model of proposed biodiesel production from palm oil.

Thailand, is Tenera (F1 Hybrid) or DxP, which derived from the hybridization between Dura and Pisifera species (Kulavanich et al., 1988; Sarakoon et al., 1998).

Among Thailand's major oil crop production, OAE (2000a) reported that, during the past decade, three major oil crops as soybean, coconut and oil palm occupy the most areas of harvested or planted. Harvested areas of soybean, coconut and oil palm in the year 1998/99 are 1.37, 2.066 and 1.129 million rais (1 rai = 0.16 ha), respectively. Nevertheless, areas for oil palm were increasing; whereas, soybean and coconut were reducing and constant, respectively. The average growth of oil palm harvested areas increased at the rate of 8.48% during the past decade (OAE, 2000b).

In term of production yield, oil palm has also shown the highest yield among these major oil crops during the past decade. The production rate was increasing at the average rate of 12.06% (OAE, 2000b). In comparison of oil seed yields and oil contents, Table 2 shows the data of the major oil crops.

Oil yield of oil palm is among the top of these major oil crops. Other researchers also reported about the percent oil content of oil palm as 21.6-24.5% by weight of a fresh fruit bunch (ffb) (Salam, 1985; Kulavanich et al., 1988). Conclusively, oil palm is quantitatively high potential for commercial plantation and production for Thailand.

Additionally, perennial crops, such as oil palm, generally consume less herbicide and impact on soil erosion than annual crops.

Chemical composition and property suitability

Biodiesel typically contains up to 14 different types of fatty acids that are chemically transformed into fatty acid methyl esters (Tyson, 2001). Palm oil's chemical compositions are within the range of those types of fatty acids (Table 3).

One parameter, which is necessary when defining general standards for biodiesel, is iodine value. Iodine value is the standard to describe and measure the degree or content of unsaturated fatty acid in vegetable oil. Iodine value is only dependent on the origin of the vegetable oil (Mittelbach, 1996; Knothe and Dunn, 2001; Lang et al., 2001). Iodine value correlates with cetane number (CN), which is used to measure of fuel ignition characteristics, like octane number for gasoline. Biodiesel from vegetable oils with low iodine value will have a higher CN while the low-temperature properties are poor. Whereas, high iodine value will have low CN while the low temperature properties are better (Knothe and Dunn, 2001). Mittelbach (1996) also stated the necessary of a limitation of unsaturated fatty acids due to the fact that heating higher unsaturated fatty acids results in polymerization of glycerides (esters). This can lead to the formation of deposits or to deterioration of the lubricating oil.

Table 2 Products and oil's yields of various plant species.

Common name	Products' yield (tones/ha)		Oil(tones/ha)
Oil palm	19.1[a]	FFB	4.8[a]
Peanut	5.0[b]	Seed	1.75-2.75[b]
Soybean	2.3[a]/3.1[b]	Seed	0.4[a]/0.403-0.775[b]
Coconut	1.0[a]	Copra	0.625[b]
Castor bean	5.0[b]	Seed	1.75-2.75[b]
Sesame	1.0[a]	Seed	0.45-0.50[b]
Rape seed	3.0[a]	Seed	1.2[a]

Sources: [a] Mattsson et al., 2000; [b] Modified El Bassam, 1998

In German biodiesel standard E DIN 51606 regulated the maximum value of 115 g Iodine/100g (see Table 4). Typically, palm oil was reported its various iodine values of 35-61 (Knothe and Dunn, 2001); 45-60 (TIS, 1978) and 44-58 (Salunkhe *et al.*, 1992), respectively. Therefore, palm oil is generally suitable as biodiesel production feedstock (Soybean oil was excluded from this standard).

It was believed that some general parameters, like density, cetane number (CN) and content of sulfur, mainly depend on the choice of vegetable oil and cannot be influenced by different production methods or purification steps (Mittelbach, 1996). Nevertheless, Lang *et al.* (2001) found that the density of the biodiesel is influenced by the original crude oil and the refining steps to make the product. However, recently, the results of many researches reported on some improvement in CN of original vegetable oils after transesterified to biodiesel (Knothe and Dunn, 2001; IEM, 2001; Altin *et al.*, 2001; Crabbe *et al.*, 2001).

Suitably available land for oil palm plantation in Thailand

In the Thailand agricultural statistic on production and marketing reported in OAE (2000b), where showed the balance sheet of Thailand's palm oil from 1991-2000, the quantities of product and local consumption almost balanced. As the results, to avoid the conflict with food feed stock demand, it is necessary to evaluate the possibility to enhance the commercially suitable plantation areas apart from existing harvested areas for food.

Sarakoon *et al.* (1998) had done the study on analysis and classification of the suitably available land in 14 provinces within the southern of Thailand (included Prachuap Khirikhan) for oil palm plantation. These areas were excluded forest, existing plantation and communities or residential areas, the summary was shown as the follows;

• Suitable land, means the production capability of 3 tons-ffb/rai/yr; (L1), equals 12,971,928 rais

Table 3 Weight percent of fatty acids in palm oil and kernel oil with typical 14 fatty acids in biodiesel.

14 Fatty acids (Tyson, 2001)	Carbon & double bond	Palm oil % fatty acid content				Palm kernel oil % fatty acid content		
		(Tyson, 2001)	(Knothe and Dunn, 2001)	Malaysia (Salunkhe *et al.*, 1992)	(Kulavanich *et al.*, 1988)	(Tyson, 2001)	(Salunkhe *et al.*, 1992)	(Kulavanich *et al.*, 1988)*
Caprylic	C8					2-4	3-4	2.8
Capric	C10					3-7	3-7	3.0
Lauric	C12					45-52	46-52	48
Myristic	C14	1-6	0.6-2.4	0.5-0.8	2	14-19	15-17	17.7
Palmitic	C16:0	32-47	32-46.3	46-51	43	6-9	6-9	8.2
Palmitoleic	C16:1					0-1		
Stearic	C18:0	1-6	4-6.3	2-4	7	1-3	1-3	1.7
Oleic	C18:1	40-52	37-53	40-42	39	10-18	13-19	16
Linoleic	C18:2	2-11	6-12	6-8	9	1-2	0.5-2.0	1.6
Linolenic	C18:3							
Arachidic	C20:0					1-2		
Eicosenoic	C20:1							
Behenic	C22:0					1-2		
Euricic	C22:1							

Remark: * reported that kernel oil also has another 1% of caproic.

• Moderate suitable land, means the production capability of 2.5-3 tons-ffb/rai/yr; (L2), equals 10,181,494 rais

• Total additionally potential land available equals 22,078,387 rais (1 rai = 0.16 ha)

The results showed that Surathani (~ 4 million rais) and Nakorn Si Thamarat (~ 3.5 million rais) have large available suitable land remaining, respectively. (Figure 2.)

To substitute 20% of today diesel fuel consumption, as stated earlier, it would require at least 2,993 Ml/year of biodiesel. Normally, the yield of methyl esters conversion process is around 95 % (see transesterification process), density of palm oil is around 0.9 kg/l (TIS, 1978; Salunkhe *et al*., 1992) and % oil content in ffb is around 21.6 % (Salam, 1985). Therefore, it would require at least 4.4 million rais of oil palm plantation areas for this purpose.

Conversion Processes

Before starting going into conversion processes to transform ffb and its oil to biodiesel, normally, there are questions on why not using crude oil in stead. Since, Rudolf Diesel's invention of the compression ignition (diesel) engine over 100 years ago, it had been known that the engine could operate on vegetable oils. Although, petroleum became the dominant world energy source, some interested researchers have been developing vegetable oil as diesel fuel source during the past century (Raneses *et al*., 1999). For example, several researches reported the using neat vegetable oils and/or the use of it blends of the oils with both direct or indirect diesel engines could cause severe numerous engine-related problems, while short term tests were almost always positive. Conclusively, long-term use of neat vegetable oils can lead to severe engine problems, emission and storage such
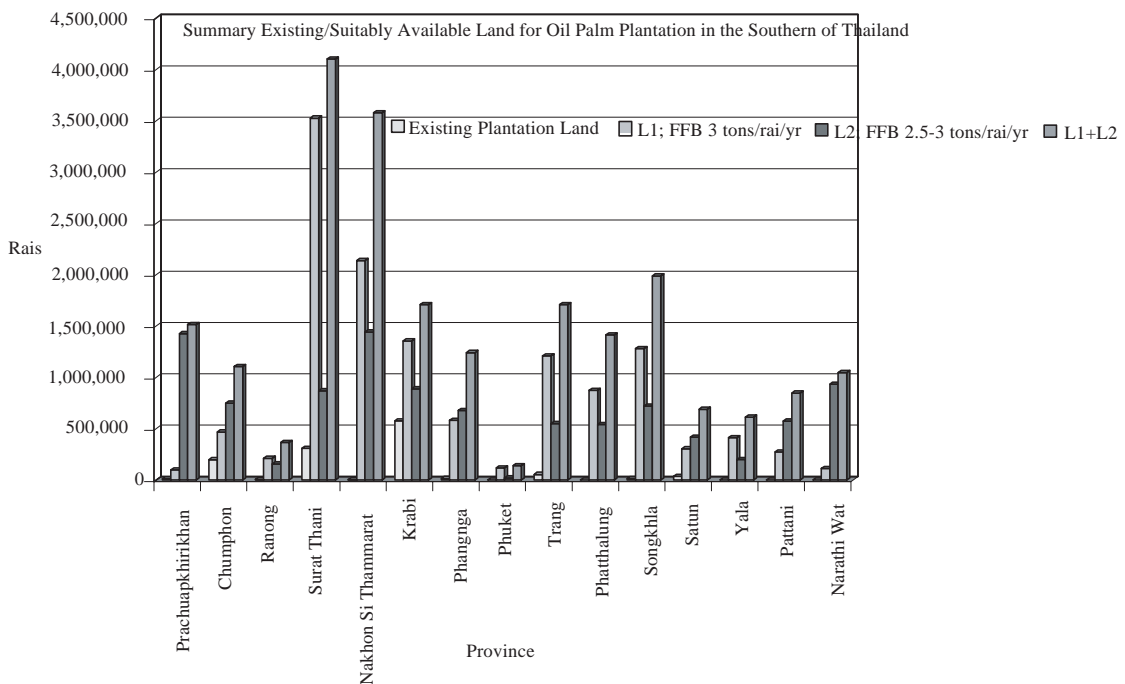

Figure 2 Summary of existing and suitably available lands for oil palm plantation among studied provinces (Modified Sarakoon *et al*., 1998)

as (Muniyappa *et al.*, 1996; Noureddini *et al.*, 1998; Canakci and Van Gerpen, 1999; Ma and Hanna, 1999; Ranesses *et al.*, 1999; Monyem *et al.*, 2001; Knothe and Dunn, 2001; Altin *et al.*, 2001);

• Coking and trumpet formation on the injectors to such an extent that fuel atomization does not occur properly or is even prevented as a result of plugged orifices,

• Carbon deposits,

• Oil ring sticking,

• Thickening and gelling of the lubricating oil as a result of contamination by the vegetable oils,

• Tendency to polymerization within the cylinder,

• Incomplete combustion,

• Triglyceride in vegetable oils can lead to formation of aromatics via acrolein from the glycerol moiety, this is able to cause PAHs known as carcinogens,

• Polymerization and gum formation caused by oxidation during storage

• Increase particulate emissions.

Bari *et al.* (2002) studied on the effects of preheating of crude palm oil (CPO) on injection system, performance and emission using Yammar L60AE-DTM single cylinder, four-stroke, air-cooled diesel engine. The results released that the suitable heating temperature in the CPO tank was 80°C enable to lower the viscosity and smoother flow with no effect to injection systems; otherwise, some effects from surplus higher temperature from heating fuel and combustion chamber during running might damage the injection pump and caused significant changes in friction between the moving parts. Moreover, the study found that CO and NO_x as emissions were higher over the whole range, compared with that of diesel, by an average value of 9.2 and 29.3%, respectively.

In order to reduced or eliminate the problems on using neat vegetable oil, it is very important to be improved, especially, viscosity, volatility and flow properties of relative triglyceride molecule in

vegetable oils. Schuchardt *et al.* (1998) concluded alternative ways, which had been considered to reduce the high viscosity of vegetable oils. Among all these alternatives, in which were dilution (25%), microemulsions, thermal decomposition, catalytic cracking and transesterification, transesterification is confirmed as the most appropriate available technology for producing monoesters, as known as biodiesel, from crude vegetable oils. Moreover, biodiesel as diesel fuel substitute, can replace diesel fuel without causing harmful effects to unmodified engines, while simultaneously reducing most of harmful exhaust emissions, especially PAHs (Polycyclic Aromatic Hydrocarbon Compounds) as known as mutagens and carcinogens, except NO_x (Gervasio,1996; Van Dyne *et al.*, 1996; Muniyappa *et al.*, 1996; Noureddini *et al.*, 1998; Sheehan *et al.*, 1998; Canakci and Van Gerpen, 1999; Connemann and Fischer, 1999; Ma and Hanna, 1999; Tat *et al.*, 2000; Krahl *et al.*, 2001; Crabbe *et al.* 2001; IEM, 2001; Knothe and Dunn 2001; Monyem *et al.*, 2001).

Oil mill factory in Thailand

Kulavanich *et al.* (1988) reported the information regarding the palm oil mill factory in the southern of Thailand. There are 14 standard factories, which have capacity from 10 to 30 tons of ffb/hr. Ten years later, Sarakoon *et al.* (1998) reported that there are 18 large factories (separated process) with total capacity as 765 tons of ffb/hr. Within these 18 factories, their capacity varies from 25 to 90 tons of ffb/hr; in addition, others 24 factories are considered small, with all together capacity is around 143 tons of ffb/hr. However, in 1997, total oil palm product (ffb) was able to cover only 53 % (year-average) of all oil mill factories' capacity.

Therefore, the total remaining capacity of these oil mill factories is around 2.05 M tons of ffb/y (based on 16 working hrs/day, 300 working days/year), which is equivalent to around 442,465 tons of crude palm oil (CPO) and crude palm kernel oil

(CPK)/y. If this remaining capacity is able to provide crude oil for biodiesel production, it will be equivalent to 1.28 Ml of biodiesel/day. This amount of biodiesel production can compensate only 3.12 % of diesel fuel consumption (41 Ml/day).

Transesterification

Transesterification is the process to transform triglyceride molecules into smaller, straight-chain molecules. These straight-chain molecules are very similar to diesel (Van Dyne *et al.*, 1996; Muniyappa, *et al.*, 1996). (Nevertheless, there are some chemically different in molecular structure and composition between biodiesel (methyl ester) and diesel. Normally, diesel molecule, as a hydrocarbon compound, has no oxygen, its general chemical formula is $C_{16}H_{34}$ or CH_3-$(CH_2)_{14}$-CH_3. On the other hand, vegetable oil molecule contains oxygen, its general chemical formula is $C_{18}H_{22}O_2$. Molecule of vegetable oil is esters of fatty acids combined with glycerol. These molecules are therefore called as aclyglycerol or glyceride, which most of them are triglyceride.). Transesterification reaction comprises of an alcohol and a triglyceride molecule in the presence of a base or acid catalyst (Noureddini *et al.*, 1998). Normally, transesterification is explainable as the displacement of the alcohol from an ester by another alcohol in a process similar to hydrolysis except that an alcohol is used instead of water. This reaction is more specifically called alcoholysis; for instance, if methanol is used, the reaction will be termed methanolysis (Gervasio, 1996).

Moreover, both Noureddni *et al.* (1998) and Knothe and Dunn (2001) stated that transesterification is currently the most common

and effective method or process for transformation of the triglyceride molecules into smaller, straight-chain molecules, reducing the high viscosity of fats and oils to a range close to that of conventional DF. The general equation of transesterification shows in Figure 3.

Regarding the stated general equation, theoretically calculated molecular weight of palm oil triglycerides and palm methyl esters (based on data of various glyceryl structures, methyl esters, and percent acid content from Sawyer and McCarty (1978); Kulavanich *et al.* (1988) and Knothe and Dunn (2001), respectively), are 865.82 and 284.18, respectively. Molecular weight of methanol and glycerine are 96 and 100, respectively. As the results, balance of the equation shows the estimated total weight ratio between triglyceride and methyl ester is almost 1:1.

Methanol usually use as an alcohol in transesterification because of its lower price than other alcohols (Ma and Hanna, 1999; Lang *et al.*, 2001; Knothe and Dunn, 2001). A conversion of 90-99% is usually obtained from this reaction depend on the process conditions. The main process conditions, which influence the conversion rate, are temperature, agitation, excess methanol (molar ratio), wt.-% catalyst, type of catalyst, reaction time, amount of water in oil, and amount of free fatty acid in oil. Practically, molar ratio of alcohol to triglycerides is higher than its stoichiometry; normally, the process has been done on the molar ratio of 6:1 or more. The reaction can be catalyzed by alkalis, acids or enzymes. The alkalis include NaOH, KOH, carbonates and corresponding sodium and potassium alkoxides such as sodium methoxide, sodium propoxide and sodium butoxide. Sulfuric
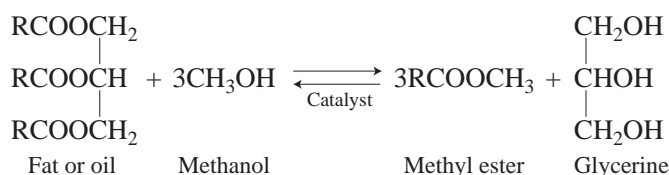

Figure 3 General equation of transesterification (Methanolysis), (Gervasio, 1996; Ma and Hanna, 1999).

acid, sulfonic acids and hydrochloric acid are usually used as acid catalysts. Lipases also can be used as biocatalysts. Alkali-catalyzed transesterification is much faster than acid-catalyzed transesterification and is most often used in commercial scales (Muniyappa *et al.*, 1996; Noureddini *et al.*, 1998; Canakci and Van Gerpen, 1999; Ma and Hanna, 1999; Crabbe *et al.*, 2001).

There are several patents on transesterifica tion of biogenic oils and fats during the past 50 years (Connemann and Fischer, 1999). Mainly, these technologies are able to divide into two groups. One is batchwise and the other is continuous process. However, the continuous process is normally well suited for large capacity requirements and using unrefined oils as feedstock. Furthermore, the unit can be designed to operate at various pressure or temperature or at atmospheric pressure and slightly temperature (Gervasio, 1996). Ma and Hanna (1999) concluded some more benefits gain from continuous process as lower the production costs, shorter reaction time, greater production capacity, more recovery of high quality glycerol, less water presented in the system, more concentrated glycerol, and lower energy required.

Currently, there are 85 biodiesel plants around the world. Within this number, there is one in Malaysia using palm oil as feedstock (Demirbas, 2000). The process of this pilot plant is two steps continuous; esterification and transesterification (esterification is the reaction of an acid with an alcohol in the presence of a catalyst to form an ester and water (Gervasio, 1996)). Malaysia's first proposed an annual capacity of 500,000 tons of palmdiesel with carotene and vitamin E recovery facilities is estimated to require an investment of RM 438 million or around Bt 5,313 million (IEM, 2001).

Biodiesel quality

Because of the fact that biodiesel is produced in quite differently scaled plants from vegetable oils of varying origin and quality, it was necessary to install a standardization of fuel quality to guarantee engine performance without any difficulties. Generally, the parameters, which are selected and established to define the quality of biodiesel, can be divided into two groups. One group contains general parameters, which are also used for mineral-oil-based fuels, and the other group especially describes the chemical composition and purity of fatty acid methyl esters. Consequently, several countries in Europe did establish standards for biodiesel such as Austria (Ö-NORM C1190/1191), Czech Republic (CSN656507), France, Germany (DIN E 51606, Table 4), Italy (UNI10635) and Sweden (SS155436); while, in USA, ASTM provides ASTM PS 121 (Table 5) as the standards to ensure good fuel quality for both pure biodiesel (B100) and blended 20% of biodiesel and 80% diesel (B20), (Mittelbach, 1996; Knothe and Dunn, 2001; Tyson, 2001).

Environmental impacts

Several researchers, such as Tat *et al.* (2000); Monyem *et al.* (2001); Krahl *et al.* (2001), Knothe and Dunn (2001) and Tyson (2001), accomplished and reported the experiments on various diesel engines in order to find the significant effects regarding methyl esters from various vegetable oils (e.g. rape seed and soybean, etc.). The results can be summarized as follows;

• Biodiesel can significantly reduce environmental impacts on PAHs as a mutagenicity compared to DF (World Health Organisation, has concluded that mineral diesel fuel is probably carcinogenic (Williamson and Badr, 1998))

• Biodiesel emissions on hydrocarbon, particulate and CO are less than DF2

• With larger hydrocarbon molecules of biodiesel are less compressible than smaller molecules. Less compressible fuels can cause early injection timing, and this can produce higher combustion pressures and temperature, which in turn produce higher NO_x emissions.

Moreover, Tyson (2001) and Altin (2001) reported the tailpipe emission changes with biodiesel

fuel produced from soybean. The results of higher blends can provide significant emission reduction benefits for carbon monoxide, particulate, soot, smoke intensity, hydrocarbons, and especially, PAHs. Körbitz (1999) also reported on some significant locally impacting emissions as summarized in Table 6.

In term of the biodegradability of biodiesel in an aquatic environment, El Bassam (1998) concluded that all of the biodiesel fuels were "readily biodegraded" compounds according to Environmental Protection Agency (EPA) standards, and have a relatively high biodegradation rate in an aquatic environment. Biodiesel can promote and speed up the biodegradation of faster the degradation rate. The biodegradation pattern in a blended biodiesel/diesel is that microorganisms metabolize both biodiesel and diesel at the same time and at almost the same rate.

Engine test performance

Although, as stated earlier, many researchers

Table 4   German biodiesel standard E DIN 51606 (Knothe and Dunn, 2001).

Fuel Property	Unit	Test method	Limit (min)	Limit (max)
Density at 15°C	g/ml	DIN EN ISO 3675	0.875	0.900
Kinematic viscosity at 15°C	mm^2/s	DIN EN ISO 3104	3.5	5.0
Flash point closed cup, Pensky-Martens	°C	DIN EN ISO 22719	110	
CFPP (cold-filter plugging point)				
-April 15-September 30	°C	DIN EN 116		0
-October 1-November 15				-10
-November 16-February 28				-20
(leap year including February 29)				
-March 1-April 14				-10
Sulfur content	wt.-%	DIN EN ISO 24260 or		0.01
		DIN EN ISO 14596		
Carbon residue	wt.-%	DIN EN ISO 10370		0.05
Cetane number		ISO/DIS 5165 : 1996 or	49	
		DIN 51773		
Ash	wt.-%	DIN 51575		0.03
Water	mg/kg	ISO/DIS 12937: 1996 or		300
Total contamination		DIN 51777-1		
Copper strip corrosion	mg/kg	DIN EN ISO 51419		20
(3h at 50°C)		DIN EN ISO 2160		1
Oxidative stability, induction time	h	IP 306***		tobe defined
Acid number	mg KOH/g	DIN 51558-1		0.5
Methanol	wt.-%	E DIN 51608		0.3
Monoacylglycerols	wt.-%			0.8
Diacylglycerols	wt.-%	E DIN 51609		0.4
Triacylglycerols	wt.-%			0.4
Free glycerols	wt.-%	To be defined		0.02
Total glycerols	wt.-%	To be defined		0.25
Iodine value	g Iodine/100g	DIN 53241-1		115
Phosphorus	mg/kg	DIN 51440-1		10
Alkali content (Na+K)	mg/kg	To be develop from DIN 51797-3, complemented by potassium		5

Table 5 Selected fuel properties for diesel and biodiesel fuels (Tyson, 2001).

Fuel Property	Diesel	Biodiesel
Fuel Standard	ASTM D975	ASTM PS 121
Fuel composition	C10-C21 HC	C12-C22 FAME
Lower heating value, Btu/gal	131,295	117,093
Kin. viscosity,@40°C	1.3-1.4	1.9-6.0
Specific gravity kg/l @ 60°F	0.85	0.88
Density, lb/gal @ 15°C	7.079	7.328
Water, ppm by wt	161	0.05% max
Carbon, wt%	87	77
Hydrogen, wt%	13	12
Oxygen, by dif. wt%	0	11
Sulfur, wt%	0.05 max.	0.0-0.0024
Boiling point, °C	188-343	182-338
Flash point, °C	60-80	100-170
Cloud point, °C	-15 to 5	-3 to 12
Pour point, °C	-35 to –15	-15 to 10
Cetane number	40-55	48-65
Stoichimometric air/fuel ratio wt./wt.	15	13.8
BOCLE Scuff, grams	3,600	>7,000
HFRR, microns	685	314

Table 6 Emission changes with biodiesel fuels.

Emission	Tailpipe emission changes (Tyson, 2001)		Locally impacting emission (Körbitz, 1999)
	B100[a] (%)	B20[b] (%)	(%)
CO	-43.2	-12.6	-20
HC	-56.3	-11.0	-32
Particulates	-55.4	-18.0	-39
NO_x	+5.8	+1.2	Slight increase[d]
Air toxics	-60 to –90	-12 to –20	ND
Mutagenicity	-80 to –90	-20	ND
CO_2	-78.3[c]	-15.7[c]	-3.2 kg/1 kg of biodiesel[e]
SO_x	ND	ND	-99
Soot	ND	ND	-59

[a]   average of data from 14 EPA FTP Heavy Duty Test Cycle tests, variety of stock engines
[b]   average of data from 14 EPA FTP Heavy Duty Cycle tests, variety of stock engines
[c]   life cycle emissions
[d]   with delay of injection timing however a decrease of 23% can be obtained
[e]   the reduction of greenhouse gaes by at least 3.2 kg CO_2-equivalent per 1 kg biodiesel

confirmed that biodiesel could substitute diesel fuel in unmodified diesel engines both direct and indirect injections with no significant effects. There are some reports, which was reviewed by Knothe and Dunn (2001) summarized as the follows;

• In numerous on-the-road tests, primarily with urban bus fleets, vehicles running on blends of biodiesel with conventional DF (80% of DF with 20% of biodiesel) required only about 2-5% more of the blended fuel than of conventional fuel. No significant engine problems were reported

• Methyl and ethyl esters of soybean oil were evaluated by 200h EMA (Engine Manufacturers Association) engines tests and compared to DF2

• Even at low blend level (≤ 2 wt%), biodiesel could serve not only as a fuel component but as a lubricity-improving additive. (Conventional DF serves as its own lubricant within the fuel system; otherwise, at low sulfur levels, this ability is lost).

In Malaysia, Schäfer (1998) and IEM (2001), reported the biodiesel quality produced from crude palm oil and palm kernel using two stage-continuous esterification and transesterification processes. The results did show that the products' quality is comparable with Malaysian diesel, especially viscosity (0.04 @40°C (ASTM D445, cST)) and pour point (16.0°C (ASTM D97)); otherwise, it didn't completely meet the DIN 51606. Its cetane number (CN) was 62.4 for pure palm oil methyl esters (POME), which is higher than European DF2; nevertheless, most engine manufacturers designate a range of required CN, usually from 40 to 50, for their engine (Knothe and Dunn, 2001). Moreover, the results of a long-term operation, both bench test and field trials (OM 352 engines), which had been done on 30 buses, 10 on 100% of this POME, 10 on 50% blended and 10 on DF2, for more than 300,000 km-each was successfully done without any major problems from this alternative fuels. Consequently, the some analysis results are,

• Modification of conventional diesel engine is not required

• The engines run smooth and are easy to start with no knocking

• Exhaust gas emission is cleaner with reduction of HC, CO, CO_2, and SO_2

• Fuel consumption is comparable to petroleum diesel e.g. 3-4 km/l for buses tested.

In the work of Körbitz (1999) mentioned on a large fleet tests done in 1990, using biodiesel from farmers' cooperative commercial production, led to engine guarantees by most of tractor producers as e.g. John Deere, Ford, Massey-Ferguson, Mercedes, Same. Later on, the year 1996, biodiesel produced from large industrial scale plants in France and Germany with the latest biodiesel standard DIN E 51606 was the basis for warranties given by major diesel engine producers such as Volkswagen, Audi, Ford, IVECO, John Deere, Kubota, MAN, Mercedes-Benz, Seat, Skoda, and Volvo.

RESULTS AND DISCUSSSION

Based on the above information, the following results and discussion can be drawn;

1. Thailand's imported crude oil reached 102 Ml/day worth Bt 285,862 million per annum in 2000. If the country were able to substitute the amount of imported crude oil for 20% of diesel consumption, it would be equivalent to at least Bt 13,436 million per annum. This amount of money will therefore circulate within the country.

2. Biodiesel is not only the renewable energy sources but also does less harmful emissions than diesel fuel, specially, on CO_2, SO_2 and PAHs. Moreover, its biodegradability can conduct the benefits on aquatic environment.

3. In term of social, economic and environmental aspects in comparison among fossil, nuclear and biomass, biomass has taken the advantages over the others.

4. Thailand's benefits form biodiesel production system can provide more incomes, jobs, products' price stability for both agricultural sectors

and rural communities, reduce risk on health, currency exchange, reliance on foreign oil and environment impacts. Biodiesel can also increase country's energy security and enhance and emphasize the country's research and development for renewable energy resources in order to prepare for the fossil energy depletion in the future.

5. Oil Palm is quantitatively the highest production yield and increasing on harvested/ plantation areas' rate over other major oil crops in Thailand. Chemical compositions and properties of both CPO and CPK are suitable as biodiesel's feedstock.

6. In case of 20% diesel substitute, biodiesel production would require at least 4.4 million rais for oil palm plantation. There are suitably available lands for oil palm plantation (excluded existing plantation, residential, community and forest areas) as 12,971,928 rais remains in 14 southern provinces (including Prachuap Khirikhan) of Thailand.

7. Using either neat vegetable oils or blended with diesel in both direct or indirect injections, although, short term tests are almost always positive, for long term uses can lead to severe engine problems, emissions and storage.

8. Although, oil mill industry's capacity in Thailand remains almost 47% by year-average, this is able to provide biodiesel only 3.12% of diesel consumption per day.

9. Transesterification is the most commercially appropriated available technology to transform crude vegetable oil to biodiesel. Oils and fats as feedstocks for advanced continuous transesterification would be limited only on water and fatty acid contents.

10. Biodiesel (from transesterification process) is acceptable to substitute diesel fuel in unmodified diesel engines without any significant effects.

11. Many industrialized countries have developed standards for biodiesel in order to guarantee engine performance without any difficulties. Notably, some parameters in these standards are significantly different depend on the environmentally utilization conditions and raw materials in each country.

12. Malaysian crude palm methyl esters had satisfied the field tests either unmodified bus diesel engine or its emissions. The first commercial biodiesel production in Malaysia was proposed an investment of RM 438 million or equivalent to around Bt 5,313 million for 500,000 tons/year.

CONCLUSION

As the results, potential biodiesel production from palm oil for Thailand is highly positive; however, the price of extracted oil palm is higher than the final competing product, petroleum diesel. Therefore, the ways to minimized cost of biodiesel is to review taxation system, develop the market of its high value co-product (glycerin) and improve the industrial crop yield and management. Currently, in UK, the market price of glycerin is £ 1300 tonne^{-1} for purified product, and is predicted to be between £ 1000 and 1300 tonne^{-1} in the year 2004 (William son and Badr, 1998). Nevertheless, within this initial stage, another market for biodiesel would be a fuel additive because of more stringent regulation on sulfur content in diesel fuel. Biodiesel's high lubricity and CN properties are comparable with today's diesel-fuel additives, which their prices are normally substantially higher than petroleum diesel itself.

LITERATURE CITED

Altin, R., S. Çetinkaya, and H.S. Yücesu. 2001. The

potential of using vegetable oil fuels as fuel for diesel engines. Energy Conversion and Management. 42 : 529-538.

Bari, S., T.H. Lim, and C.W. Yu. 2002. Effects of preheating of crude palm oil (CPO) on injection system, performance and emission of a diesel engine. Renewable Energy 27 : 339-351.

Berger, K.G. 1984. Diversification of palm oil applications, pp. 199-221. *In* Proceedings of the International Seminar on Market Development for Palm Oil Products. 23-27 January 1984. Palm Oil Research Institute of Malaysia.

Canakci, M. and J Van Gerpen. 1999. Biodiesel Production via Acid Catalysis. Transaction of ASAE 42(5) : 1203-1210.

Chambers, K. 2000. Bioenergy: 21st Century fuel, pp.11-16. *In* Guide to UK Renewable Energy Companies 2000. dti, Jame x Jame, UK.

Connemann, J. and J. Fischer. 1999. Biodiesel processing technologies and future market development, pp.1-12. *In* Paper presented at the symposion "Biodiesel-Fuel from Vegetable Oils for Compression-ignition Engines" at the TAE. May 17, 1999. Ostfildern/Stuttgart.

Cook, E.W. 1984. Diversification of palm oil applications, pp. 215-221.*In* Proceedings of the International Seminar on Market Development for Palm Oil Products. 23-27 January 1984. Palm Oil Research Institute of Malaysia.

Crabbe, E., C. Nolasco-Hipolito, G. Kabayashi, K. Sonomoto, and A. Ishizaki. 2001. Biodiesel production from crude palm oil and evaluation of butanol extraction and fuel properties. Process Biochemistry 37: 65-71.

Demirbas, A. 2000. Biomass resource facilities and biomass conversion processing for fuels and chemicals. Energy Conversion and Management 42 : 1357-1378.

El Bassam, N. 1998. Energy Plant Species, Their Use and Impact on Environment and Development. James and James (Scienece Publishers) Ltd. UK. 321 p.

Gervasio, G.C. 1996. Fatty acids and derivatives from coconut, pp. 33-56. *In* Y.H Hui (ed.).

Bailey's Industrial Oil and Fat Product. Vol. 5. Industrial and Consumer Nonedible Products from Oils and Fats. John Wiley and Son, Inc. NY.

Institution of Engineers Malaysia (IEM). 2001. Palm Diesel Time to Trap a Unique Biofuel. (cover story). JURUTERA Bulletin 8-10.

Knothe, G and R. O. Dunn. 2001. Biofuel derived from vegetable oils and fats, pp. 106-150. *In* F. D. Gunstone, and R. J Hamilton (eds.). Oleochemical Manufacture and Applications. Sheffield Academic Press. CRC.

Körbitz, W. 1999. Biodiesel production in Europe and North America, An Encouraging Prospect. Renewable Energy, 16 : 1078-1083.

Krahl, J., K Baum, U. Hackbarth, H.-E. Jeberien, A. Munack, C. Schutt, O. Schroder,N. Walter, J. Bunger, M.M. Muller, and A. Weigel. 2001. Gaseous compounds, ozone precursors, particle number and particle size distributions, and mutagenic effects due to biodiesel. Trans ASEA 44(2) : 179-191.

Kulavanich, P., S. Klinpikul, S. Kulavanich, and S. Chiramanee. 1988. Transformed Products and Marketing Development Project of Small Palm Oil Milling Regarding HM The King's Patronage. Prince of Songklanakarin University, Songkla. 127 p. (in Thai).

Lang, X., A.K. Dalai, N.N. Bakhshi, M.J. Reaney, and P.B. Hertz. 2001. Preparation and characterization of bio-diesel from various bio-oils. Bioresource Technology 80 : 53-62.

Ma, F. and A. M. Hanna. 1999. Biodiesel production: a review. Bioresource Technology 70 : 1-15.

Mattsson, B., C. Cederberg, and L. Blix. 2000. Agricultural land use in life cycle assessment (LCA): case studies of three vegetable oil crops. Journal of Cleaner Production 8 : 283-292.

Mittelbach, M. 1996. Diesel fuel derived from vegetable oils, VI: Specifications and quality control of biodiesel. Bioresource Technology 56 : 7-11.

Monyem, A., J.H. Van Gerpen, and M. Canakci. 2001. The effect of timing and oxidation on emissions from biodiesel-fueled engines.

Transaction of ASAE 44(1) : 35-42.

Muniyappa, P.R., S.C. Brammer, and H. Noureddini. 1996. Improved Conversion of Plant Oils and Animal Fats into Biodiesel and Co-product. Bioresource Technology 56 : 19-24.

Noureddini, H., D. Harkey, and V. Medikonduru. 1998. A Continuous Process for the Conversion of Vegetable Oils into Methyl Esters of Fatty Acids. JAOCS. 75(12) : 1775-1783

Raneses, A.R., L.K. Glaser., J.M. Price, and J.A. Duffield .1999. Potential biodiesel markets and their economic effects on the aricultural sector of the United States. Industrial Crops and Products 9 : 151-162.

Salam, B. A. 1985. The contribution of the oil palm by-products towards the Nationnal Energy Policy-an economist point of view, pp. 133-145. *In* Proceedings of the National Symposium on Oil Palm By-Products for Agro-Based Industries. 5-6 November 1985. Palm Oil Research Institute of Malaysia, Kuala Lumpur.

Salunkhe, D.K; J.K Chavan.; R.N. Adsule and S.S. Kadam. 1992. World Oilseeds, Chemistry, Technology and Utilization. AVI Book. New York. 545 p.

Sarakoon, N., S. Sinthurahat, and S. Dansakulphon. 1998. Agro-ecological Zoning Analysis for Oil Palm in the South of Thailand. Department of Agriculture. The Ministry of Agriculture and Cooperatives. Bangkok. 266 p. (in Thai.)

Sawyer, C.N. and P.L. McCarty. 1978. Chemistry for Environmental Engineering. 3rd ed, McGraw-Hill. New York. 532 p.

Schäfer, A. 1998. palm oil fatty acid methyl ester (POME) as diesel fuel in Malaysia, pp. 23-43. *In* Proceedings of the 1998 PORIM International Biofuel and Lubricant Conference, 4-5 May 1998, Malaysia.

Schuchardt, U., R. Sercheli, and R.M. Vargas. 1998. Transesterification of vegetable oils : a Review, *J. Braz. Chem. Soc*. 9(1) : 199-210.

Sheehan, J., V. Comobreco, J. Buffield, M. Graboski, and H. Shapouri. 1998. Life Cycle Inventory of Biodiesel and Petroleum Diesel for Use in Urban Bus. Final Report. prepared for USDOE and USDOA. National Renewable Laboratory. USA. 285 p.

Tat, M.E., J.H. Van Gerpen, S. Soylu, M. Canakci, A. Monyem, and S. Wormley. 2000. The speed of sound and Isentropic bulk modulus of biodiesel at 21°C from atmospheric pressure to 35 Mpa. JAOCS, 77(3) : 285-289.

The National Energy Policy Committee (NEPC). 2001. NEPC's Minute of Meeting 3/2544. NEPO. Thailand. 8p.

The National Energy Policy Office (NEPO). 2001.Thailand's Energy Situation 2001. NEPO Journal 53 : 53-113.

The Office of Agricultural Economics (OAE). 2000a, Agricultural Statistics of Thailand Crop Year 1998/99. Thailand. 161 p. (in Thai)

The Office of Agricultural Economics (OAE). 2000b. Information on the Important Agricultural Productions and Marketing. Statistical document no. 7/2543. Thailand. 167 p. (in Thai)

TIS 288-2521. 1978. Standard for Palm Oil. Thailand Industrial Standard Institute. 11p. (in Thai)

Tyson, S.K. 2001. Biodiesel Handling and Use Guidelines. National Renewable Laboratory. U.S. Department of Energy. NREL/TP-580-30004. CO. USA. 17p.

Van Dyne, D.L., J.A. Weber, and C.H. Braschler. 1996. Macroeconomic effects of a community-based biodiesel production system. Bioresource Technology 56 : 1-6.

Williamson, A-M. and O Badr . 1998. Assessing the viability of using Rape Methyl Ester (RME) as an alternative to mineral diesel Fuel for Powering Road Vehicles in the UK. Applied Energy. 59(2-3) : 187-214.

Statistical Analysis of Influenced Factors Affecting the Plastic Limit of Soils

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ABSTRACT

The determination of plastic limit of soils according to ASTM Standards specifies the value of plastic limit as the moisture content of rolled soil thread at 3.2 mm diameter that begins to crumble. The reliability of test results must depend on the skill of operator and various mechanical factors. In practice, test results of plastic limit have a high variation. In this research, influenced factors on the variation of results were studied. Statistical analysis of results by multiple regression, correlation, and analysis of variance indicated that there were two primary factors affecting the plastic limit of soils. The main factor was the initial size of the soil sample, explained 40% variation of the plastic limit value. The second factor was the type of soil classified by plasticity explained 21.8 % variation of the value. The other mechanical factors such as friction, speed, and pressure explained 3.0-3.2 % variation of the plastic limit value of soils.

Key words: plastic limit, statistical analysis, soil testing, correlation

INTRODUCTION

The Atterberg's limits are useful in agricultural soil science and soil engineering. They also correlate with some important engineering properties of soil. The Atterberg's limits, which are most useful for engineering purposes are liquid limit, plastic limit and shrinkage, limit. These limits are expressed as percent water content. Atterberg (1911) defined the plastic limit as the water content at which a sample of soil begins to crumble when rolled into a thread under the palm of the hand (Casagrande, 1932). In order to standardize the test, Terzaghi (1926) set the diameter of the thread at 3.2 mm or 1/8 inch. Mechanically, this procedure subjects the soil to a very complex stress system in that it combines bar rolling distortion, cylindrical compression and lateral extrusion process. A rigorous analysis for these stresses does not exist.

However, assuming full saturation and incompressibility of soil mass, plasticity theory indicates the soil yield stress to be functions of applied pressure to the soil bead, geometry of soil sample, speed of rolling and friction between soil, hand and base plate (White, 1982). None of these variables are controlled in the rolling bead test, and the known variation of results is not surprising. For example, the slightly cohesive clay in this research with mean plastic limit 28.42%, when tested by different operators, the results range from as low as 22% to as high as 34%.

In this research, statistical techniques such as multiple regression, correlation analysis, and analysis of variance were applied to identify which influenced factors are the main factors that affect the plastic limit variation. In order to parametrically control each influenced factors, a mechanical rolling device was modified from Bobrowski and

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Griekspoor (1992). The influenced factors considered in this study are applied pressure to the soil bead, geometry of soil sample, speed of rolling, and friction among soil, hand and base plate.

MATERIALS AND METHODS

Mechanical rolling device design

The mechanical rolling device was developed from Bobrowski's rolling device as shown in Figure 1. At the interior intersection between the two sides and the base, a plexiglass rail 3.2 mm high was placed. These rails will accurately dictate the exact diameter of the soil thread. In order to obtain the adjustable speed of the test, a DC motor(1) with adapter(2) was connected to the upper plate to produce the rolling action. Variation of the input voltage from 16.5 to 19.5 volts yields the rolling speed of 103 to 128 cycle/min. The plexiglas plate(3) with bolt poles attached was used to control the pressure that apply to the soil bead.
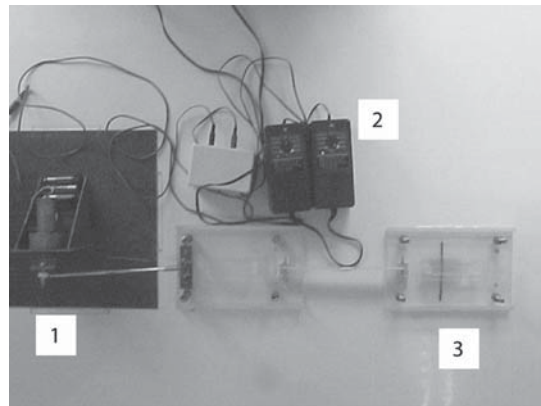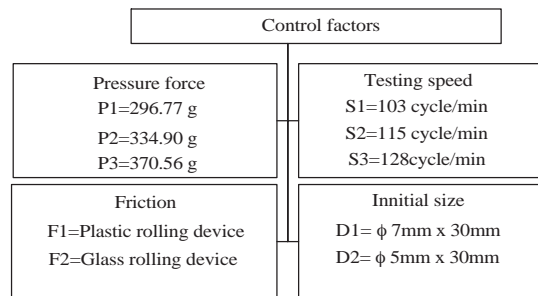
Materials and experimental design

In this research, three representative samples of soils that are very cohesive soil, moderately cohesive soil, and slightly cohesive soil were prepared for testing. Figure 2 shows the variation of control factors used in the experimental design plan.

The rolling bead test cannot be expected to provide reliable and consistent results for plastic limit since none of variables are controlled in the rolling bead test. In this experimental design plan, rate of deformation (R_d) was set to be the representative variable demonstrating the rate of shape changing due to the bar rolling distortion, cylindrical compression, and lateral extrusion process in the plastic limit test as shown in Figure 3.
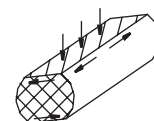
The rate of deformation (R_d) was defined as

$$R_d = \frac{(L_1 / D_1 - L_0 / D_0)}{t}$$

Figure 1 The mechanical rolling device.

Figure 2 The variation of control factors used in experimental design plan.
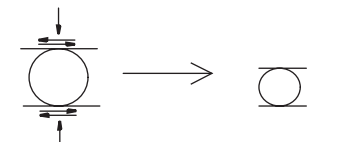
(a) Rolling distortion process
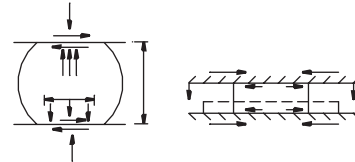
(b) Cylindrical compression process

(c) Lateral extrusion process

Figure 3 Plastic limit testing as a mechanical process.

In which

     R_d = rate of deformation (sec^{-1})
     L_0 = initial length of sample (mm)
     D_0 = initial diameter of sample (mm)
     L_1 = final length of sample (mm)
     D_1 = final diameter of sample (mm)
     t   = testing time (sec)

The rate of deformation was calculated in various conditions (Table 1). The analyses of results by multiple regression, correlation, and analysis of variance were applied to identify which factors are the primary factors affecting the plastic limit variation.

RESULTS AND DISCUSSION

From a total of 216 tests, the plastic limit values and the rate of deformation were determined. Statistical analyses were carried out using the Minitab and SPSS package, which include multiple regression, correlation analysis, and analysis of variance. In this way, factors affecting the results can be identified separately (Lyman, 1993). The

results from analyses were demonstrated in Figure 4 and Table 2. From the illustration, it can be noticed that

1. Rate of deformation will increase if the applied pressure and rolling speed is increased.

2. Rate of deformation will increase when the initial diameter decreases (From D1 to D2).

3. Rate of deformation of very cohesive soil (soil type 1) is greater than the moderate (soil type 2) and slightly cohesive soil (soil type 3).

4. Rate of deformation in coarse surface rolling device (plastic plate) is greater than the smooth rolling device (glass plate).

The results analyzed by multiple regression and correlation in Table 2 indicated that there were two primary factors affecting the plastic limit. The main factor was the initial size of the soil sample, explained 40% variation of plastic limit value. The second factor was the type of soil classified by plasticity explained 21.8 % variation of the value. The other mechanical factors such as friction, speed, and pressure explained 3.0-3.2 % variation of plastic limit value.

In order to ensure the statistical analysis

Table 1 Testing condition for R_d in experimental plan.

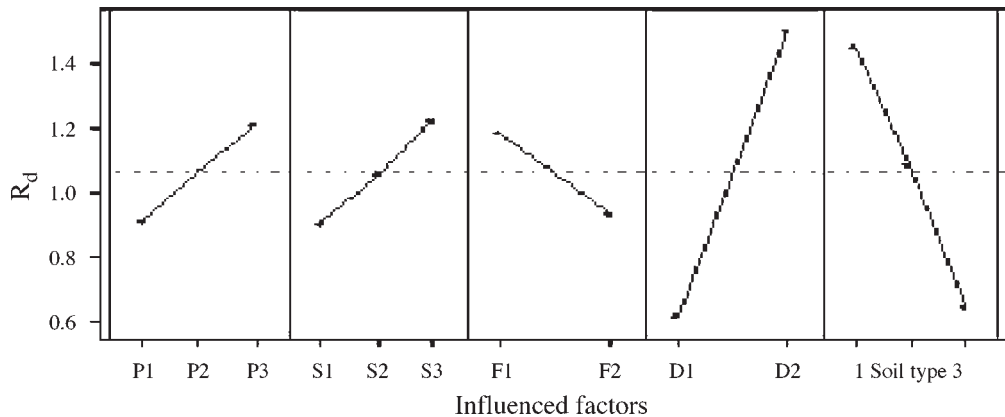| Rolling device material	Initial size of sample	Testing time (sec)	Pressure force (g)	Testing speed (cycle/min)		
				S1	S2	S3
	ϕ 7mm × 30mm		296.77 (P1)	103	115	128
Translucent plastic	(D1)	15	334.90 (P2)	103	115	128
(Coarse surface, F1)			370.56 (P3)	103	115	128
	ϕ 5mm × 30mm		296.77 (P1)	103	115	128
	(D2)	5	334.90 (P2)	103	115	128
			370.56 (P3)	103	115	128
	ϕ7mm × 30mm		296.77 (P1)	103	115	128
Plexiglass	(D1)	15	334.90 (P2)	103	115	128
(Smooth surface, F2)			370.56 (P3)	103	115	128
	ϕ 5mm × 30mm		296.77 (P1)	103	115	128
	(D2)	5	334.90 (P2)	103	115	128
			370.56 (P3)	103	115	128

Figure 4 Relation between rate of deformation and influenced factors from analysis of variance.

Table 2 Results of multiple regression and correlation analysis by Stepwise method.

Model	R	R Square	Adjusted R Square	Incremental Adjusted R Square (%)
1	0.635	0.403	0.400	40.0
2	0.789	0.622	0.618	21.8
3	0.809	0.655	0.650	3.2
4	0.829	0.688	0.682	3.2
5	0.848	0.718	0.712	3.0

Note   Model 1: R_d = f (**Diameter**)
Model 2: R_d = f (Diameter 1, **Soil type**)
Model 3: R_d = f (Diameter 1, Soil type, **Speed**)
Model 4: R_d = f (Diameter 1, Soil type, Friction 1, **Friction**)
Model 5: R_d = f (Diameter 1, Soil type, Friction 1, Speed 1, **Pressure**)
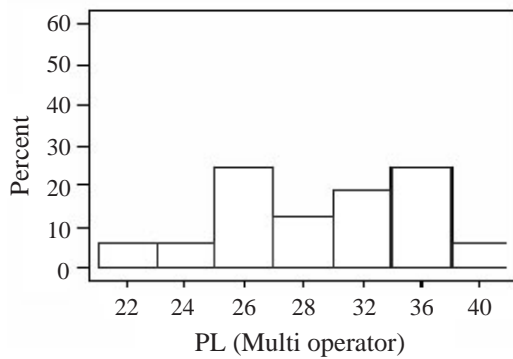
results, variation of plastic limit value before and after controlling main influenced factors were determined in Figure 5. From the figure, it can be noticed that the range of plastic value reduces from 22-34 % to 28-30%.
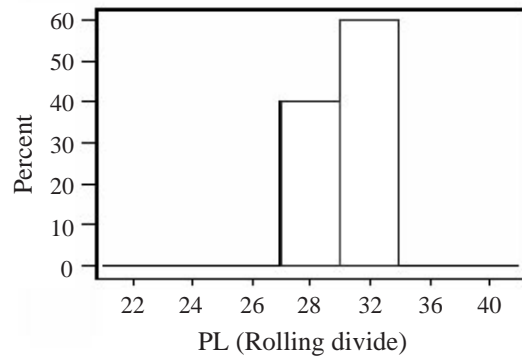
CONCLUSION

The analysis from multiple regression, correlation, and analysis of variance of the plastic limit test results with various controlling factors and soil types, found that there were two primary factors affecting the plastic limit of soils. The main factor was the initial size of the soil sample, explained 40% variation of plastic limit value. The second factor was the type of soil classified by plasticity explained 21.8 % variation of the value. The other mechanical factors such as friction, speed, and pressure explained 3.0-3.2 % variation of plastic limit value.

The results of the plastic limit test with controlling main influenced factors demonstrated a significantly reduction in variation. It can be recommended that the plastic limit test could be

(a) Before controlling influenced factors.   (b) After controlling influenced factors.

Figure 5   Variation of plastic limit results before and after controlling the main influenced factors.

standardized to give more reliable and repeatable results by the initial diameter of the soil thread sample.

LITERATURE CITED

Atterberg, A. 1911. Uder die physikalische Bondenuntereuschung and uber die Plastizitat der tone, International Miteilungen fur Budenkunde. Vol.1, No 5.

Bobrowski, L.J. and D.M. Griekspoor. 1992. Determination of plastic limit of soil by mean of a rolling device. Geotechnical Testing J. 15 (3) : 284-287.

Casagrande, A. 1932. Research on the Atterberg's limit of soils. Public Road 13 : 121-130.

Lyman, O.R. 1993. An Introduction to Statistical Methods and Data Analysis, Duxbury Press.

Terzaghi, K. 1926. Simplified soil tests for subgrades and their physical significance. Public Road 7 : 153.

White, I.L. 1982. Soil plasticity and strength: a new approach using extrusion. Ground Engineering 15 : 16-24.

Determination of Selenium in Water Samples by Using a Methylene Blue Kinetic Catalytic Spectrophotometric Method

Apisit Songsasen[1], Pasit Aukkarayunyong[1], Sornarin Bangkedphol[1] and Wantana Sasomsap[2]

ABSTRACT

A kinetic catalytic spectrophotometric method, which was very sensitive and effective, was developed for the determination of selenium in water samples. It based on the catalytic effect of selenium on the reaction of methylene blue with sodium sulfide. A change in the absorbance of methylene blue with times at various concentrations of selenium were monitored, giving the "end point" (time) for each concentration of selenium. A plot of end point versus selenium concentration constituted a calibration graph, which was linear in a range of 2.5-30 ppb of selenium, with the correlation coefficient of 0.9992. The method was applied to determine the amount of selenium in the water sample containing 15 ppb of selenium, giving 91.84% recovery and the relative standard deviation of 2.27%. Compared with the hydride generation atomic absorption spectrometry method, this method is more sensitive for the determination of selenium in the water sample when the concentration of selenium is lower than 100 ppb.

Key words: selenium, methylene blue, spectrophotometric method, water sample

INTRODUCTION

Selenium is essential to life. It shares many properties of sulfur and arsenic. Its compounds are covalent existing in several allotropic forms including Se_8. The oxide dissolves in diluted bases to give selenites such as Na_2SeO_3. Although it is an essential nutrient in small amounts, selenium and its compounds are toxic at slightly high levels. Elemental selenium is widely used in electronic semiconductors, as it conducts electricity in the light, and hexavalent selenium occurs widely as selenate in natural waters. The acute oral dose LD_{50} of sodium selenite in rats is 7 mg/kg, and that of sodium selenate is 4 mg/kg, with its the principal action affecting the nervous system (Crosby, 1998).

Selenium has been determined by many methods but the most common method is hydride generation atomic absorption spectrometry (Cassella *et al.*, 2002; Bujdos *et al.*, 1999). The hydride generation atomic absorption spectrometry (HGAAS) method is sensitive but the experimental equipment is not readily available in many laboratories. However, spectrophotometer which is much cheaper and easier to operate, is readily accessible in most laboratories.

There are several spectrophotometric methods for the determination of selenium (Afsar *et al.*, 1989) but most of them have limited sensitivity and often high detection limit (ppm to sub-ppb) which can not be used to detect selenium in the samples containing selenium in the level of ppb. However, kinetic catalytic spectrophotometric

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methods have yielded better detection limits for selenium determinations than simple spectrophotometric methods (Shiundu and Wade, 1991; Mottola and Perez-Bendito, 1992). The method based on catalytic effect on a reduction of methylene blue (MB) by sodium sulfide (West and Ramakrishna, 1968) :

$$2MB + S^{2-} + 2H_2O \longrightarrow 2HMB + 2OH^- + S$$
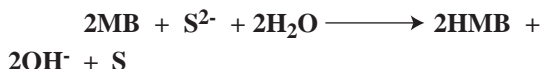
In the reduction, the colour of methylene blue is blue; after the reduction of methylene blue by sodium sulfide, the reaction gives HMB which is colourless. In the presence of excess sulfide, sulfur will combine with sulfide ions, and give the polysulfides :

$$S + S^{2-} \longrightarrow [S\text{——}S]^{2-}$$

Similarly, when selenium combines with sulfide ions, it gives selenosulfides :

$$Se + S^{2-} \longrightarrow [S\text{——}Se]^{2-}$$

Then selenosulfides react with methylene blue in a similar way to the sulfide ions:

$$2MB + [S\text{——}Se]^{2-} + 2H_2O \longrightarrow 2HMB + 2OH^- + S + Se$$
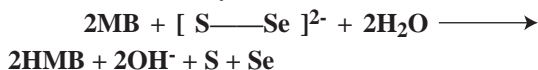
However, the selenosulfide ion reacts with methylene blue more quickly than sulfide ion and selenium is generated at the end of the reaction.

Gokmen and Abdelqader (1994) used the kinetic catalytic spectrophotometric method for the determination of selenium in urine samples (84.9 % recovery). A plot of t^{-1} (reciprocal of time at the end point) versus various concentrations of selenium was used as the calibration graph. In this study, we tried to simplify the catalytic method by using a plot of time (end point) versus various concentrations of selenium as the calibration graph which made method more convenient to use in laboratories. The catalytic method was also tried to be used in analyzing a very low concentration of selenium in water samples that could not be determined by using the HGAAS methods because of high detection limit.

MATERIALS AND METHODS

Reagents

All reagents used were analytical reagent grade and their solutions made up in deionized water. Formaldehyde solution (assay 37%) was purchased from BDH (Poole, England).

Preparation of solutions

- Preparation of selenium standard solution

Selenium atomic absorption stock solution (1000 ppm Merck, Darmstadt, Germany) was used to prepare Se(IV) (10 ppm) by diluting the stock solution with 1% HNO_3 (Malinckrodt, Kentucky, USA). The selenium standard solution (10 ppm) was diluted to lower concentration (2.5-30 ppb) by 1% HNO_3.

- Preparation of conditioner solution

Conditioner solution was prepared by mixing 0.69 g EDTA (Merck, Darmstadt, Germany), 0.0145 g $FeCl_3$ (Merck, Darmstadt, Germany) and 1.25 ml triethanolamine $[(HOCH_2CH_2)_3N]$ (Merck, Darmstadt, Germany) together and dissolving the mixture in deionized water, then diluting to 250 ml.

- Preparation of methylene blue solution (0.05% MB)

Methylene blue solution was prepared by 0.05 g of dissolving methylene blue (Merck, Darmstadt, Germany) in deionized water, then diluting to 100 ml.

- Preparation of sodium sulfide solution

Sodium sulfide solution was prepared by dissolving 5.04 g $Na_2S.9H_2O$ (Ajax Chemical, Auburn, Australia), 4.80 g of Na_2SO_3 (Merck, Darmstadt, Germany) and 1.60 g NaOH (Carlo Erba, Val de Reuil, France) in water, then diluting to 100 ml.

- Solution of the interfering ions

Potassium solutions were prepared from KCl (Carlo Erba, Val de Reuil, France)

Calcium solutions were prepared from $CaCl_2$ (Carlo Erba, Val de Reuil, France)

Magnesium solutions were prepared from MgCl$_2$ (Carlo Erba, Val de Reuil, France)

Zinc solutions were prepared from ZnCl$_2$ (Ajax Chemical, Auburn, Australia)

Iron Solutions were prepared from FeCl$_3$ (BDH, Poole, England)

Apparatus

A double beam UV-Visible Spectrophotometer (JASCO model 7800) was used to record absorbance versus wavelength and absorbance versus time.

Experimental procedure

Each experiment was carried by adding 6.5 ml standard selenium (or samples), 1.0 ml formaldehyde, 2.5 ml conditioner solution, 0.5 ml sodium sulfide solution and 1.0 ml methylene blue solution into a beaker (The time interval for each addition was 30 seconds). Recording absorbance at wavelength 668 nm which is the absorption maxima of methylene blue (Figure 1) versus time spectra at room temperature is shown in Figure 2. The time (t) for completion of the reaction between methylene blue and sulfide were determined from the intersection of the two tangents.
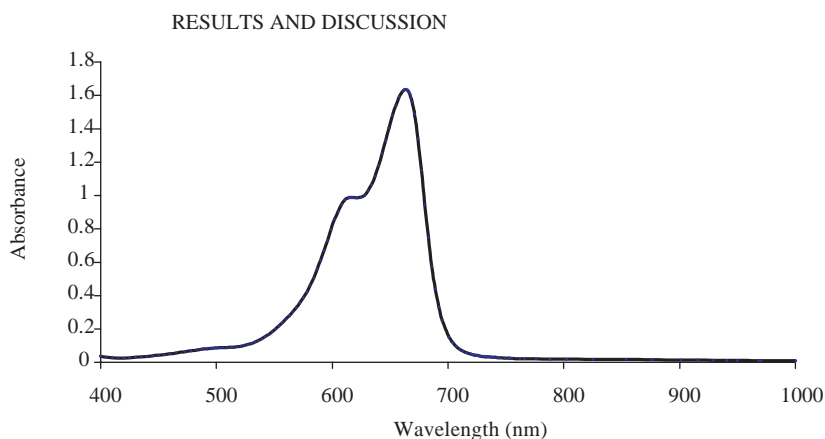
RESULTS AND DISCUSSION

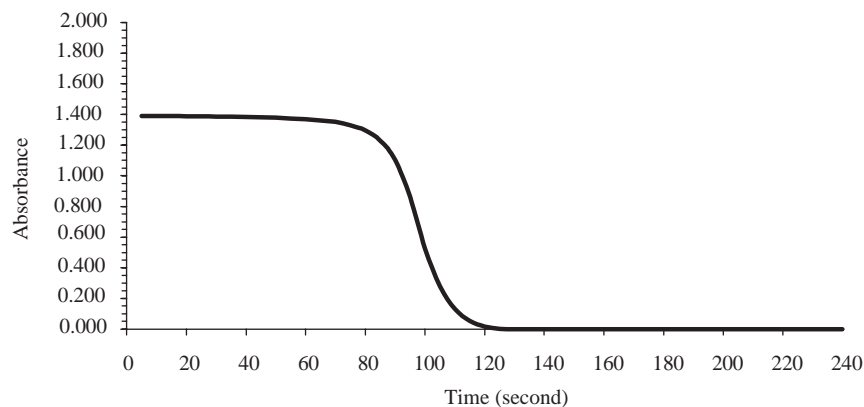**Figure 1**   Absorption spectrum of methylene blue.

Figure 2   Absorbance versus time spectrum for the mixture of standard selenium solution and methylene blue at 668 nm.

RESULTS AND DISCUSSION

Optimization of experimental parameters

In these experiments, the concentration of methylene blue, formaldehyde and conditioner solution were kept constant. Only the effect of time interval before recording the absorbance and concentration of sodium sulfide solution were investigated in order to increase the sensitivity of the catalytic method. The reason for fixing the volume of 0.05% methylene blue at 1.0 ml was that, we would like to use the method for the determination of selenium at very low concentration of water samples which could not be determined by the HGAAS method. High concentration of methylene blue in the analyzing solution could affect the accuracy of the method. For iron(III), it was reported that in the presence of Na_2H_2EDTA and sulfide, a faintly cherry-red coloured complex formed on the addition of iron(III) resulting in the removing dissolved oxygen (West and Ramakrishna, 1968)**.** This meant that iron(III) could enhance the reduction of methylene blue by selenosulfide. As Gokmen and Abdelqader's works (1994), ferric chloride was also used as part of the conditioner solution together with Na_2H_2EDTA and triethnolamine. Triethanolamine and formaldehyde do not have any effect on the catalytic reaction but may help in maintaining the higher oxidation state of iron by suppressing the reducing power of sodium sulfide on iron(III)(West and Ramakrishna, 1968). EDTA was a general masking agent to eliminate several interfering ions by complexing them and preventing their reactions with the sulfide ion.

- Effect of time interval before spectrum recording

In the experiment, all other parameters were kept constant except the time interval after mixing the solution and recording a spectrum. Figure 3 shows the adsorption spectra of selenium solutions where the time interval are 20, 40, and 60 seconds. The end point was found to change significantly with the time interval, hence fixing the time interval before recording the spectrum was very important in determining the amount of selenium in the samples. The time interval for 20 seconds was chosen for all experiments with the following reasons; firstly, for increasing in an accuracy in determining the end point from the spectrum and, secondly, for time saving.

- Effect of concentration of sodium sulfide in the analyzing solution

In this experiment, all others parameters were kept constant except the concentration of sodium sulfide solution. The volumes of sodium
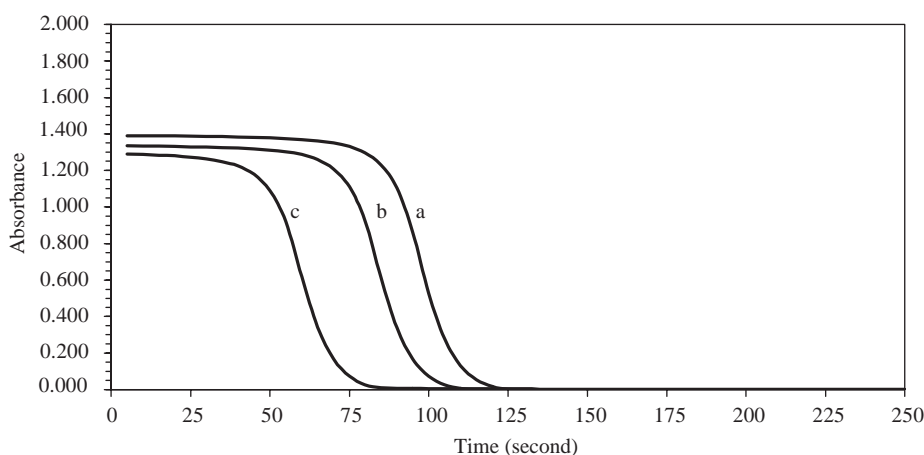

Figure 3 Absorbance versus time spectra for the analyzing solution at 668 nm when time interval before recording the spectra were (a) 20 seconds (b) 40 seconds (c) 60 seconds.

sulfide solution used were 0.5, 1.5 and 2.0 ml. The absorption spectra are shown in Figure 4. We found that the end points were changed significantly with the volume of sodium sulfide solution changed. When the concentration of sodium sulfide were high (1.5 and 2.0 ml), the accuracy in determining the end point reduced. This might be because, at high concentration, sodium sulfide exceedingly stimulated the methylene blue to HMB (colourless) and caused difficulty in determining the end point, therefore volume of sodium sulfide solution at 0.5 ml was chosen for all experiments.

Calibration graph, recovery and accuracy of the method

After studying the effect of concentration of sodium sulfide and the time interval before spectrum recording, conformity to Beer's law over the concentration of selenium was determined. Under the optimized condition, end point changed linearly with selenium concentration over the ranges of 2.5-30 ppb. The linear calibration graph with the correlation coefficient of 0.9992 is shown in Figure 5. For the water sample containing 15 ppb of selenium, it was found that the kinetic catalytic
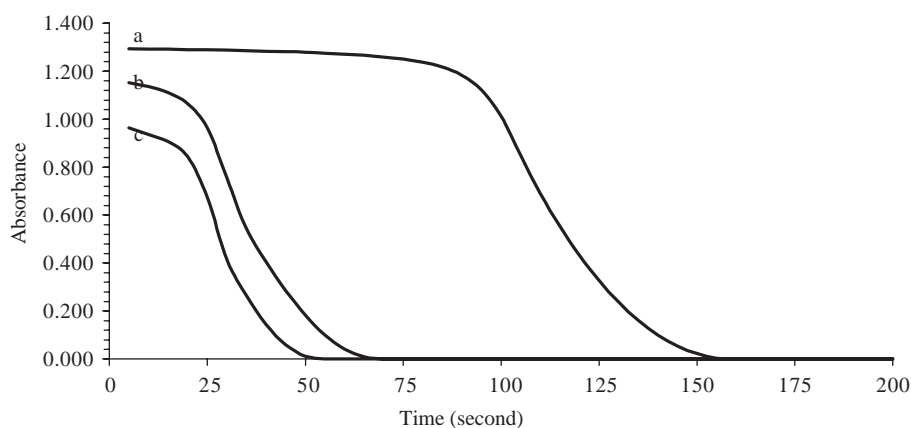

Figure 4 Absorbance versus time spectra for the analyzing solution at 668 nm when volume of sodium sulfide solution were (a) 0.5 ml (b) 1.5 ml (c) 2.0 ml.
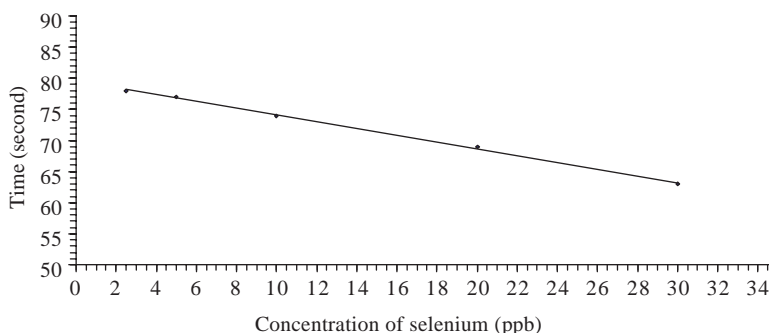

Figure 5 The calibration graph for the determination of selenium in the water sample by using the catalytic method (a plot of end point versus selenium concentration, correlation coefficient(r) = 0.9992)

spectrophotometric method gave 91.84 % recovery with the relative standard deviation of 2.27%. This meant that the catalytic method was very sensitive and effective for the determination of selenium in the samples containing selenium in the level of ppb

Interference studies

The ions chosen for interference studies were K^+, Mg^{2+}, Ca^{2+}, Zn^{2+} and Fe^{3+} which were normally present in high concentration in water samples. Different concentrations of individual interferent ions were added to the samples consisting of 15 ppb selenium. The concentration of the studied ion increased until the error in determination of 15 ppb selenium was over 2SD (SD = standard deviation, confidence limit = 95%). The tolerance

ratio was defined as the ratio of the concentration of the ion causing error over 2SD in the determination of selenium to the concentration of selenium which was calculated for each ion studied (table 1). Of the studied ions, Zn^{2+} had the lowest tolerance ratio, followed by Fe^{3+}, Ca^{2+}, Mg^{2+}, and K^+, respectively. This implied that the removal of Zn^{2+} and Fe^{3+} from the samples before the determination of selenium by this catalytic method is necessary. However, Ca^{2+}, Mg^{2+}, and K^+, seemed not to have affect on the determination of selenium by this method.

Comparison of the kinetic catalytic spectrophoto-metric method with the HGAAS method

Concentration of selenium in five unknown water samples (supplied by the analytical laboratory of Thailand Institute of Scientific and Technological Research) were determined by the catalytic method and the HGAAS method. The results are shown in table 2. The kinetic catalytic method had an advantage in determining of selenium in the water sample at the concentration lower than 0.1 ppm which is the lowest limit of the HGAAS method.

CONCLUSION

The kinetic catalytic spectrophotometric

Table 1 Tolerance ratio for various interfering ions on the determination of 15 ppb Se(IV).

Ions	Tolerance ratio
K^+	7000
Mg^{2+}	3000
Ca^{2+}	2000
Fe^{3+}	5.5
Zn^{2+}	4.5

Table 2 Comparison of the kinetic catalytic spectrophotometric method with the HGAAS method for the determination of selenium in the water samples.

Sample	HGAAS method[a]	Kinetic catalytic spectrophoto metricmethod[b]
1	< 0.1 ppm	100 ppb
2	< 0.1 ppm	70 ppb
3	< 0.1 ppm	50 ppb
4	< 0.1 ppm	25 ppb
5	< 0.1 ppm	70 ppb

a = HGAAS method was determined and reported by the Analytical laboratory of Thailand Institute of Scientific and Technological Research.

b = average value from 4 replicates.

method based on the catalytic effect of selenium on the reaction of methylene blue with sodium sulfide had a high sensitivity and accuracy in determining the concentration of selenium in water samples, with the relative standard deviation of 2.27% at 15.00 ppb standard selenium. This method is easy to use and has an advantage over the hydride generation atomic absorption spectrometric method (HGAAS) in determination of selenium at a very low concentration (< 0.1 ppm).

ACKNOWLEDGEMENT

LITERATURE CITED

Afsar, H., R. Apak, and I. Tor. 1989. Spectro-photometric determination of selenium with 2 – Mercaptoethanal. Analyst 114 : 1319-1324.

Bujdos, M., J. Kubova, and V. Stresko. 1999. Problems of selenium fractionation in soils rich in organic matter. Anal. Chem. Acta. 408: 103-109.

Cassella, R.J., A.T. Rangelc, and R.E. Santellib. 2002. Selenium determination by electrothermal atomic absorption spectrometry in petroleum refinery aqueous stremas containing volatile organic compounds. Microchemical Journal 71: 21-28.

Crosby, D.G. 1998. Environmental Toxicology and Chemistry. Oxford University Press, New York. 336 p.

Gokmen, G. and E. Abdelqader. 1994. Determination of selenium in biological metrices using a kinetic catalytic method. Analyst. 119 : 703-708.

Mottola, H.A. and D. Perez-Bendito. 1992. Kinetic determinations and some kinetic aspects of analytical chemistry. Anal. Chem. 64 : 407R-428R.

Shiundu, P.M. and A.P. Wade. 1991. Development of catalytic photometric flow injection methods for the determination of selenium. Anal. Chem. 63 : 692-699.

West, P.W. and T.V. Ramakrishna. 1968. A catalytic method for determining traces of selenium. Anal. Chem. 40 : 966-968.

Irrigation Efficiency of the Greater Chao Phraya and the Greater Mae Klong Irrigation Projects

Varawoot Vudhivanich[1] , Singha Pajongkitkran[2]
Adisak Bunpian[2] and Nimit Cherdchanpipat[1]

ABSTRACT

The irrigation efficiency of each block of the Greater Chao Phraya Irrigation Project(GCPP) and the Greater Mae Klong Irrigation Project(GMKP) were calculated on both wet and dry season during 1995-1998. GCPP was divided into 18 blocks. Each block in GCPP covered the area of one or more irrigation subproject according to the hydraulic boundary of the irrigation area such that the inflow and the outflow of the block could be measured. GMKP was divided into 10 blocks. Each block in GMKP was the same as the irrigation subproject. The Ei of GCPP varied between 14.6-55.4% with the average value of 39.4%. The Ei of GMKP varied between 24.5-51.0% with the average value of 43.2%. In general, the Ei of GMKP was about 4% higher than that of GCPP. Both GCPP and GMKP used the continuous water delivery with the upstream control practices. The Ei on both GCPP and GMKP varied considerably. Borommthart project on the upper right bank of GCPP had the highest Ei of 63.7% while Pakhai project had the lowest Ei of 13.3%. For GMKP, Song Phi Nong project on the upper left bank had the highest Ei of 66.8% while Thamaka project on the right bank had the lowest Ei of 19.2%. The wet season Ei on both GCPP and GMKP had a linear relationship with the annual rainfall. The dry season Ei was linearly related to the water available at the beginning of the dry season and the irrigated area. Besides, the irrigated area was highly correlated to the available water.

Key words: irrigation efficiency, irrigation, water management, Chao Phraya , Mae Klong

<table>
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INTRODUCTION

The Greater Chao Phraya and the Greater Mae Klong Irrigation Projects are the two largest and the most important irrigation projects in Thailand. They are located in the central plain. These two projects have the combined irrigation service area of about 1.7 million hectares (10.5 million rais) or about 40% of the total irrigation area of Thailand. The Greater Chao Phraya Irrigation Project (GCPP) is the rice bowl of Thailand while

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the Greater Mae Klong Irrigation Project (GMKP) is the main sugarcane and sugar producing area of the country.

GCPP has two big multipurpose reservoirs, Bhumiphol and Sirikit, with the combined storage capacity of 22,972 mcm. These two reservoirs supply water to GCPP and for other purposes. Normally, the water is insufficient (Kobayashi *et al*. 1994). They can supply less than half of the GCPP area in the dry season.

GCPP is the largest irrigation project in

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Thailand having the irrigation service area of 7.5 million rai. The main crop on both wet and dry season is paddy. The headworks of GCPP is the Chao Phraya Diversion Dam where water is distributed to 25 irrigation subprojects on both left and right banks. GCPP was first developed in 1957. Most of the canals are unlined. The water is distributed by continuous delivery with upstream control. The irrigation efficiencies in many irrigation subprojects are low. Rehabilitation, modernization and management improvement are needed.

GMKP is the second largest irrigation project area of Thailand having the irrigation service area of 3 million rais. Paddy is cultivated in about two third of the area and about one third cultivating sugarcane. The main source of water for GMKP comes from Srinagarind and Vajiralongkorn multipurpose reservoirs (The old name of Vajiralongkorn is Khao Laem). These two reservoirs have the total combined storage capacity of 26,605 mcm. Mae Klong Diversion Dam is the headworks of GMKP where water is diverted to the left and right main canals and distributed to about 3.0 million rais of the cultivated area in 10 irrigation subprojects. Continuous water delivery with upstream control is the method of water delivery and control practices in GMKP. Most of the canals are relatively new comparing with those of GCPP and most of them are concrete lined to reduce the conveyance losses.

Since GCPP and GMKP are the most important irrigation project of Thailand and these two projects require very large amount of irrigation water annually. It happened quite oftenly that the water is insufficient, particularly for GCPP. Besides, each irrigation project has many hundred kilometers length of the canal. High conveyance losses are usually exist. The irrigation efficiency which is one of the indicators reflecting the performance of irrigation system needs to be studied. The irrigation efficiency is useful for making decision on irrigation management improvement, rehabilitation and modernization of the old irrigation system.

Therefore, this study is conducted with the following objectives :

(1) analyze the irrigation efficiency of the GCPP and the GMKP irrigation projects.

(2) determine the factors effecting the irrigation efficiency in both irrigation projects.

MATERIALS AND METHODS

Required data

(1) Maps showing canals, drains, control structures and irrigation system boundaries of the Greater Chao Phraya Irrigation Project (GCPP) and the Greater Mae Klong Irrigation Project (GMKP).

(2) Water allocation data including crop data, agro-climatological data, daily rainfall and daily discharge at the major control structures of both projects during 1995-1998. The water allocation data were collected from 25 irrigation subprojects in GCPP and 10 irrigation subprojects in GMKP.

Methods

(1) The general water allocation and distribution methods of GCPP and GMKP were studied.

(2) The water allocation data including the daily and weekly data were collected. The daily data were the rainfall and discharge at the major control structures. The weekly data were the weekly crop data.

(3) The schematic diagram showing the canal and drainage networks, the irrigation area and the water distribution systems in GCPP and GMKP were drawn. The GCPP was divided into 18 blocks as shown in Figure 1. Each block covered one or more irrigation subprojects according to the hydraulic boundary such that the inflow and the outflow of the block could be measured. For example, the block named **Phollathep-Thaboat** covered 2 irrigation subprojects, Phollathep and Thaboat irrigation subprojects. Ten blocks were located on the right (or west) bank while the other 8 blocks were on the left (or east) bank. The GMKP was divided into 10 blocks as shown in Figure 2.
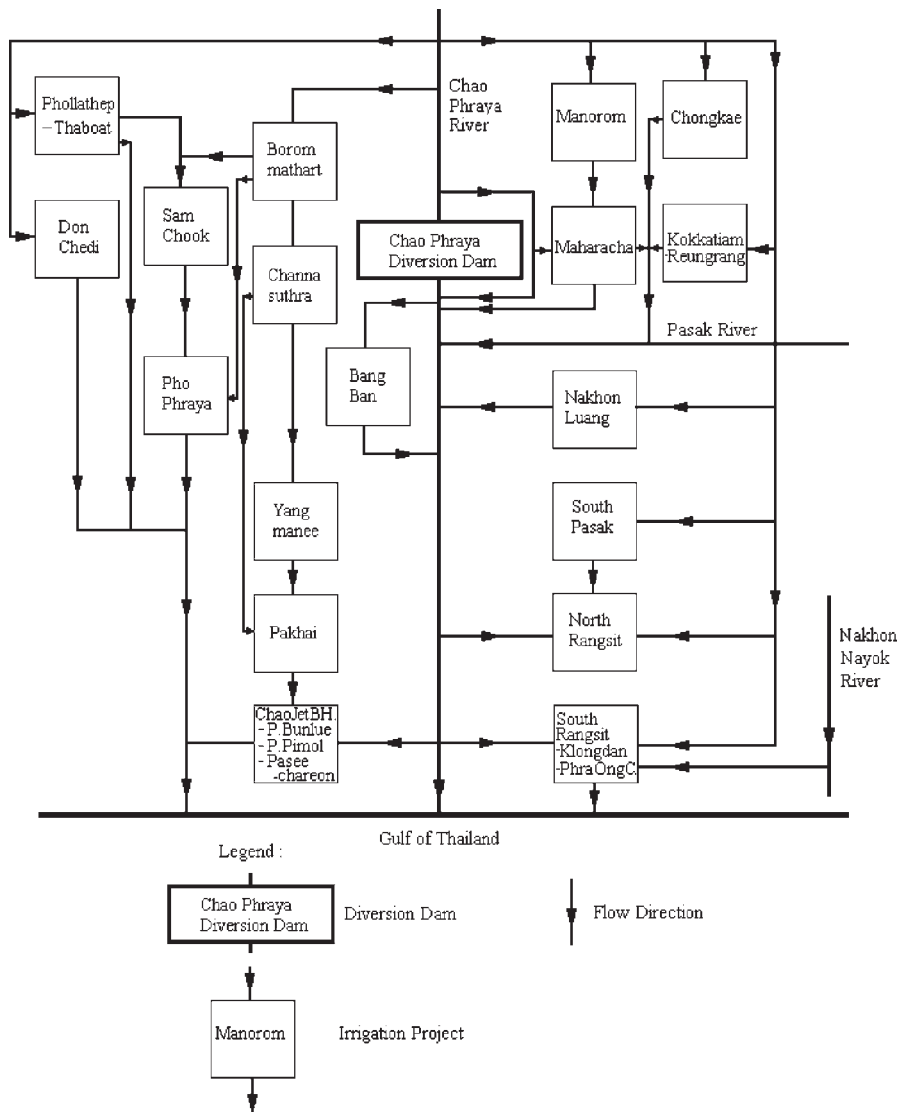
Figure 1 Schematic diagram showing water distribution system of GCPP.

Each block in GMKP was the same as the irrigation subproject. There were 8 irrigation subprojects on the left bank and 2 subprojects on the right bank.

　　　　(4) The crop water requirements (ETc = Kc. ETo) and irrigation efficiency for each irrigation subproject of GCPP and GMKP were calculated on weekly basis for both wet and dry season of 1995-1998.

　　　　(5) The factors effecting the irrigation efficiency of GCPP and GMKP were analyzed.

Theoretical considerations for irrigation efficiency evaluation

　　　　Irrigation Efficiency is usually defined as the percentage of the net irrigation requirement to the gross irrigation supply. In order to determine the irrigation efficiency of any irrigation project or system, the boundary of the irrigation system needs to be defined such that the inflow and outflow of irrigation water can be measured. In most of the irrigation subproject in GCPP and GMKP, there
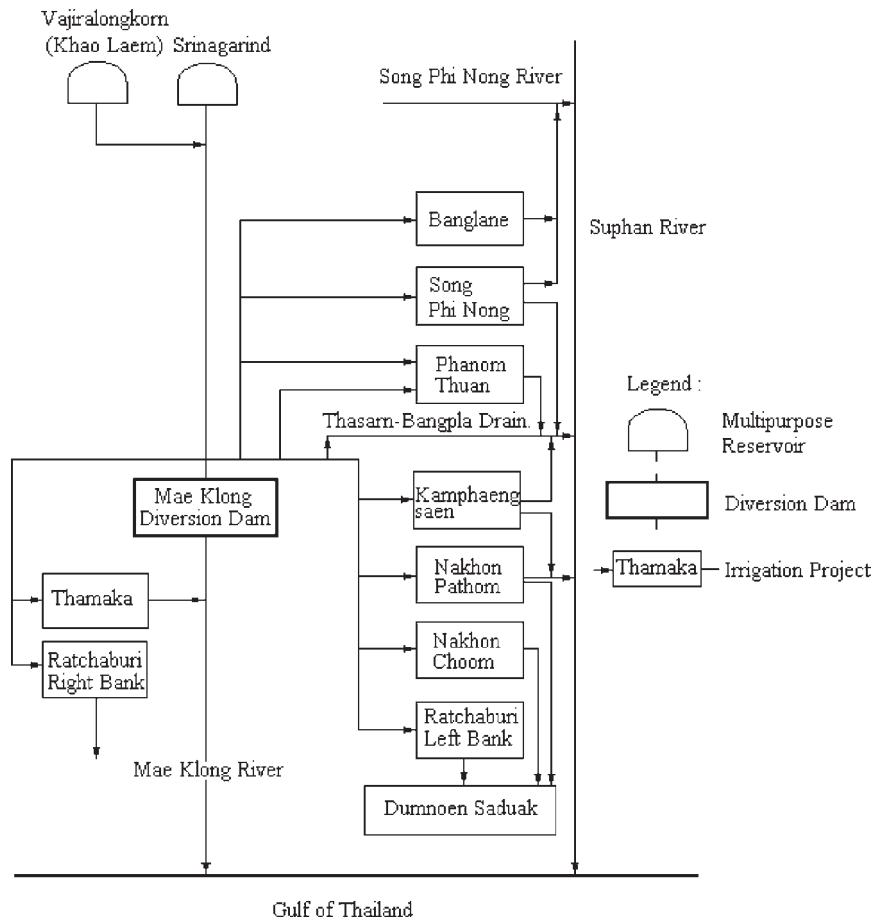
Figure 2 Schematic diagram showing water distribution system of GMKP.

are more than one inflow and outflow points. The gross irrigation supply is calculated from the total inflow minus the total outflow. Kirdpitak (1985) suggested the practical method for calculating the project irrigation efficiency on weekly basis by using the data normally collected by an irrigation project in Thailand. The formula is given below :

$$Ei\,(\%) \;=\; 100\frac{[ETc + LP + P - Re]}{Q}\; ...\,(1)$$

Where Ei   = Irrigation efficiency (%)
ETc   = Total crop water requirements (cms)
LP   = Total land preparation requirements (cms)
P   = Total percolation losses (cms)

Re   = Total effective rainfall (cms)
Q   = Total irrigation supply (cms) which equals to the total inflow minus the total outflow to any irrigation system.

RESULTS AND DISCUSSION

Irrigation efficiency of GCPP

The average of the weekly irrigation efficiency (Ei) of the wet and dry season for 16 blocks out of 18 blocks in GCPP was shown in Figure 3. Since Maharacha and Bang Ban did not have sufficient and reliable data for irrigation efficiency calculation, they were omitted from the analysis. In general, the GCPP Ei varied between
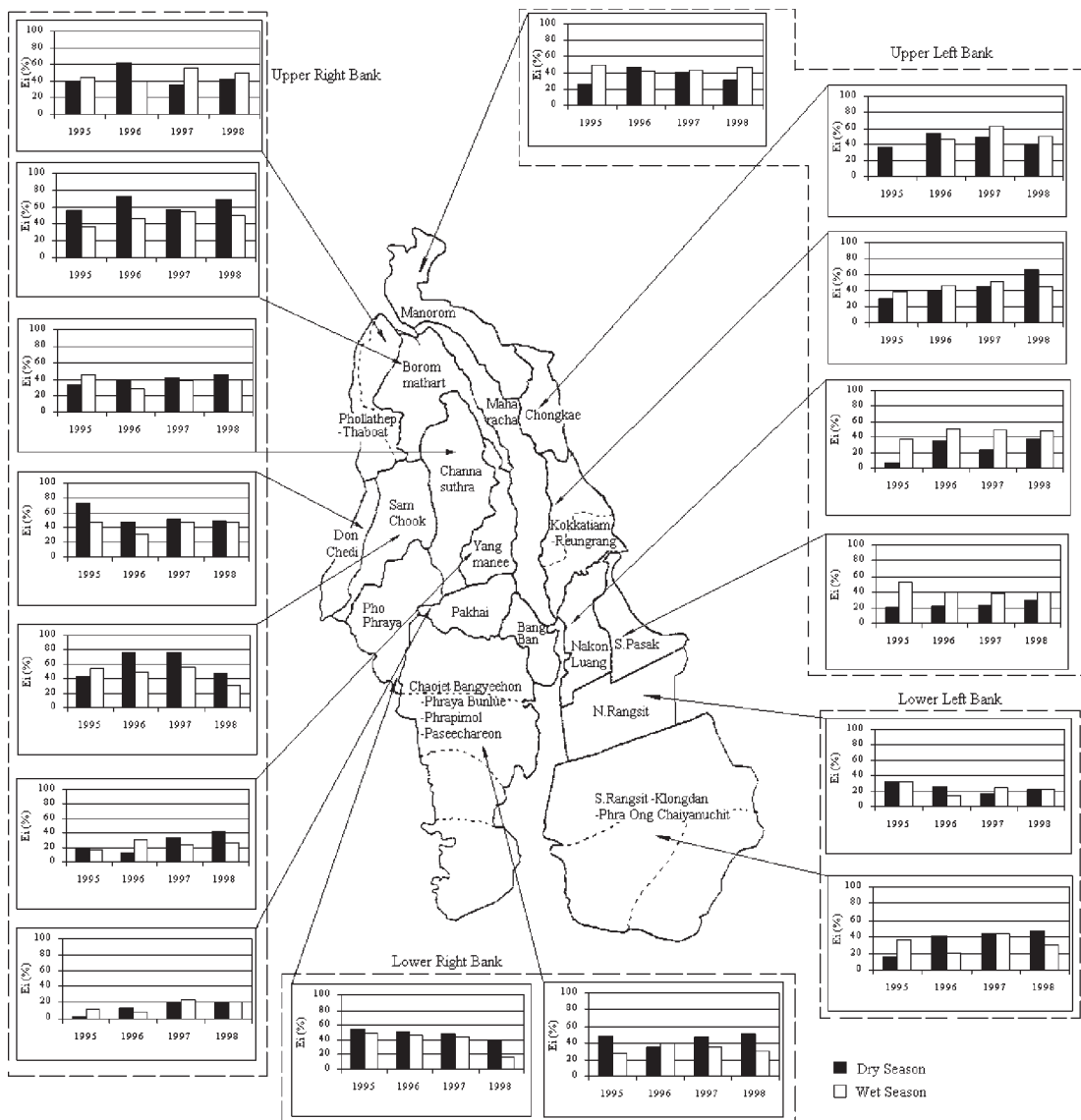
Figure 3 Irrigation efficiency of GCPP.

14.6-55.4% during 1995-1998 with the average value of 39.4%. In dry season, the GCPP Ei varied between 13.3-63.7% during 1995-1998. The 4 year average GCPP Ei was 39.7%. Borommathart irrigation project on the right bank showed the highest Ei of 63.7% while Pakhai irrigation project on the right bank showed the lowest Ei of 13.3%. In wet season, the GCPP Ei varied between 15.8-53.4% during 1995-1998. The 4 year average GCPP Ei was 39.1%, about the same as the dry season Ei.

Seven blocks on the GCPP upper right(or west) bank including Phollathep-Thaboat, Don Chedi, Sam Chook, Borommathart, Channasuthra, Yangmanee and Pakhai taking irrigation water from Makhamtao-U Thong canal, the Suphanburi river and the Noi river had Ei of 43.4% in the dry season and 36.4% in the wet season. On the left (or east) bank , 5 blocks on the upper left bank including Manorom, Chongkae, Kokkatiam-Reungrang, South Pasak and Nakhon Luang had Ei of 35.2% in

the dry season and 46.7% in the wet season. Two blocks on the lower left bank including North Rangsit and South Rangsit-Klongdan-Phra Ong Chaiyanuchit had Ei of 30.5% in the dry season and 28% in the wet season.

In general, the right bank Ei (40.7%) was higher than the the left bank Ei (37.6%).

Irrigation efficiency of GMKP

The average of the weekly irrigation efficiency (Ei) of the wet and dry season for each subproject in GMKP was shown in Figure 4. The GMKP Ei varied between 24.5-51.0% during 1995-1998 with the average value of 43.2% which was in the range of the previous study (Vudhivanich *et al.*,2000; Kanoksing *et al.*,2001; AIT,1994). In dry season, the GMKP Ei varied between 29.9-66.8% during 1995-1998. The 4 year average GMKP Ei was 48.4%. Song Phi Nong irrigation project on the upper left bank showed the highest Ei of 66.8%
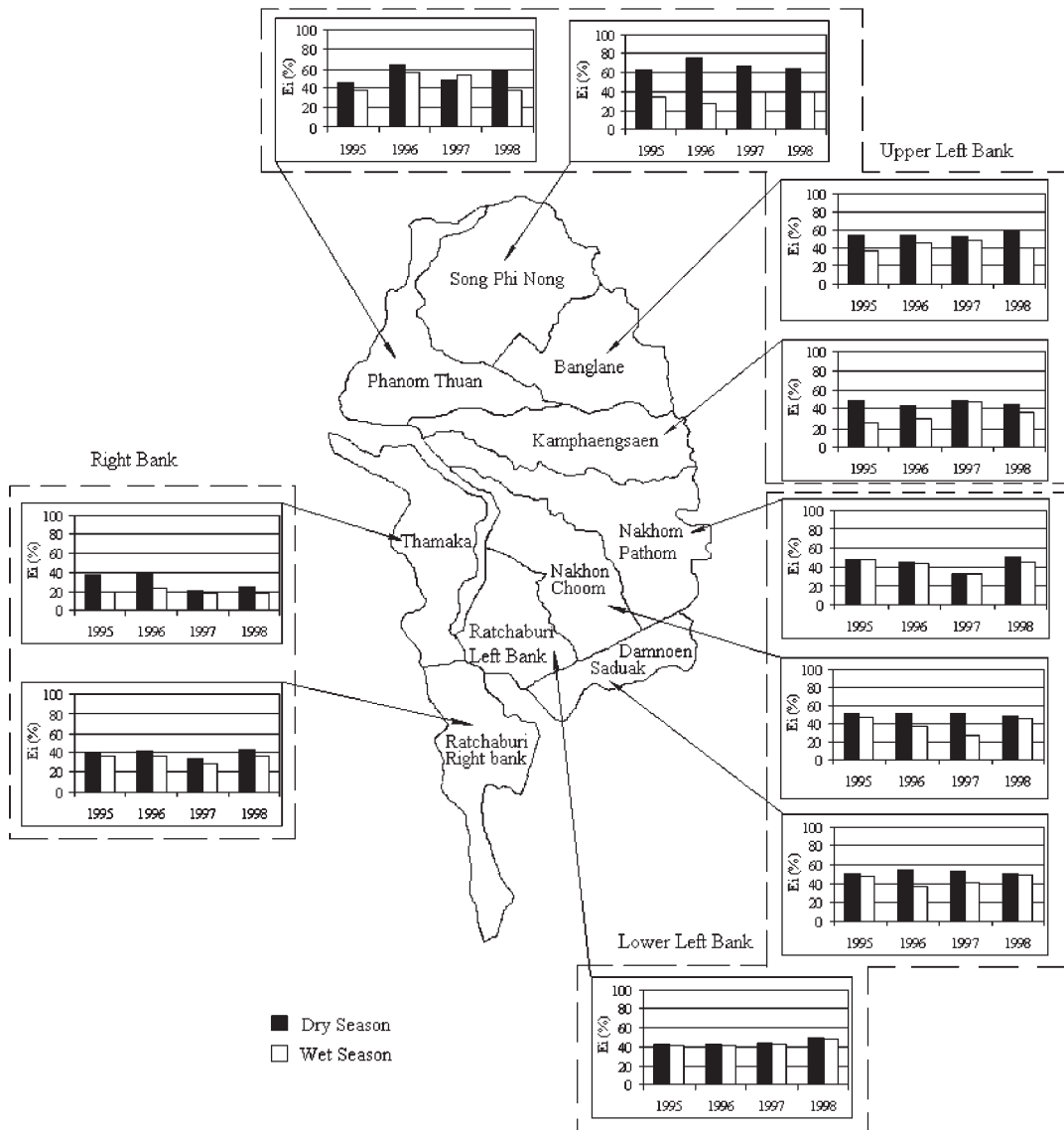

Figure 4 Irrigation efficiency of GMKP.

while Thamaka irrigation project on the right bank showed the lowest Ei of 29.9%. In wet season, the GMKP Ei varied between 19.2-46.6% during 1995-1998. The 4 year average GMKP Ei was 38.1%, about 10% lower than the dry season Ei. Phanom Thuan irrigation project on the upper left bank showed the highest Ei of 46.6% while Thamaka irrigation project on the right bank showed the lowest Ei of 19.2%. The irrigation project on the left bank had higher Ei than the project on the right bank due to the better canal water distribution system. The irrigation water on the left bank project distributed from main canal to secondary, tertiary and finally to farm land in sequent. On the contrary, some projects on the right bank system distributed water directly from main canal to tertiary system. This made water distribution control difficulty.

Factors effecting Ei of GCPP

The wet season Ei of GCPP had the linear relationship with the annual rainfall with r^2 of 0.9671 as shown in Figure 5. The wet season Ei decreased as the annual rainfall increased. This indicated that the annual rainfall had effect on the wet season Ei. This could be explained as follow. Firstly, as the annual rainfall increased, more water was available to farmers both in term of more effective rainfall in the paddy field and more water available in the Bhumipol and Sirikit reservoirs.
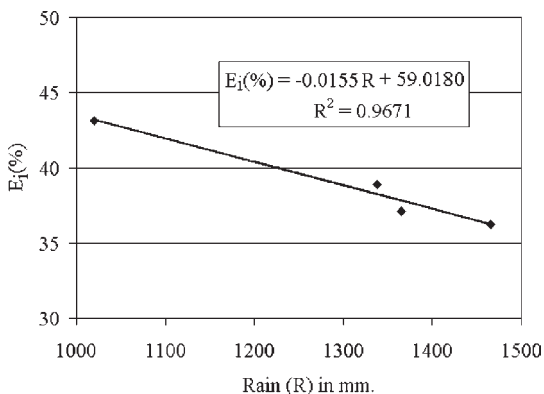

Figure 5 Effect of rainfall on the wet season Ei in GCPP.

Once the farmers and irrigation project staffs realized that water was available. They tended to be more relax on the control and use of irrigation water. The result was the lower efficiency. Secondly, the rainfall had some significant effect on Ei due to the ineffective water delivery and control methods used in GCPP. The GCPP used the upstream control water delivery system by calculating the irrigation water requirements of each canal section on weekly basis. In the calculation, the expected rainfall was estimated. If the actual rainfall was greater than the estimated value. The irrigation water would be used less efficient although each project tries to reduce the irrigation water supply by readjusting the regulators after the rainfall taking place, the water losses were already occurred.

In dry season, the GCPP Ei was linearly related to the water available in Bhumipol and Sirikit reservoirs at the beginning of dry season (W) and the irrigated area (A) as shown in Figure 6. The correlation coefficients (r) among Ei, W and A were higher than 0.9 in general. Dry season Ei was linearly related to A and W with the correlation coefficients (r) of 0.82 and 0.62 respectively. Also the W and A were highly related with r equals to 0.96. The dry season Ei of GMKP was related to A and W in similar manner as the dry season Ei of GCPP in Figure 6. Ei increased as the irrigated area and the amount of water available(W) in the reservoirs at the beginning of the dry season increased. This was due to the fact that when the available water was limited, the irrigation area was decreased. The irrigated area could not be controlled and grouped into one area for effective water distribution. It was spread widely over the irrigation project area. With this situation, high conveyance losses were taking place.

The effect of the annual rainfall, irrigated area and available water on Ei would be useful for improving the irrigation efficiency of GCPP and the irrigation subprojects in GCPP and also for other projects.
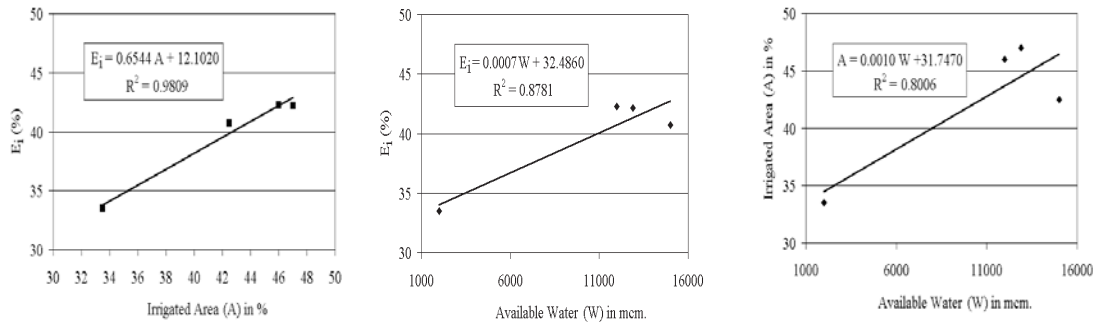
Correlation Coefficients

	Irrigation efficiency (Ei) in%	Available water (W) in mcm.	Irrigated area (A) in%
Irrigation Efficiency(Ei) in%	1		
Available Water(W) in mcm.	0.94	1	
Irrigated Area(A) in%	0.99	0.89	1

Figure 6 Effect of irrigated area and the amount of water supply at Chao Phraya diversion dam on dry season Ei in GCPP.

Factors effecting Ei of GMKP

In wet season, Ei decreased as the annual rainfall increases as shown in Figure 7. The annual rainfall was an important factor effecting wet season Ei of GMKP which was the same as the case of GCPP in Figure 5.

In dry season, the situation was different. Ei was related to 2 important factors: the irrigated area and the amount of water available (W) at Mae Klong diversion dam due to the supply from Srinagarind and Khao Laem storage dams as shown in Figure 8.

CONCLUSION

The irrigation efficiencies (Ei) during 1995-1998 varied between 14.6-55.4% with the average value of 39.4% for irrigation block in GCPP and between 24.5-51.0% with the average value of 43.2% for irrigation subproject in GMKP. For GCPP, the Ei on wet and dry season was not
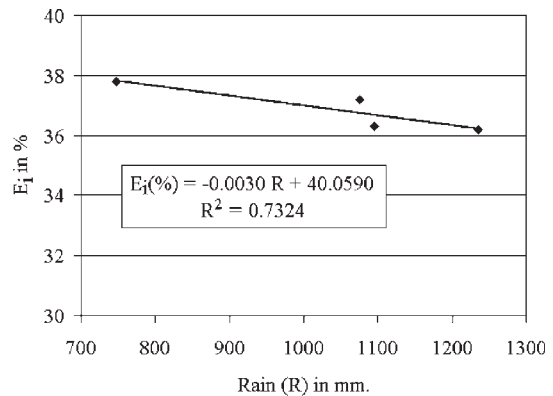
Figure 7 Effect of rainfall on wet season Ei in GMKP.

different. Borommathart project on the upper right bank showed the highest Ei of 63.7% in dry season while Pakhai project showed the lowest Ei of 13.3%. For GMKP, the dry season Ei was 48.4% on the average which was about 10% higher than the wet season Ei. Song Phi Nong project on the upper left
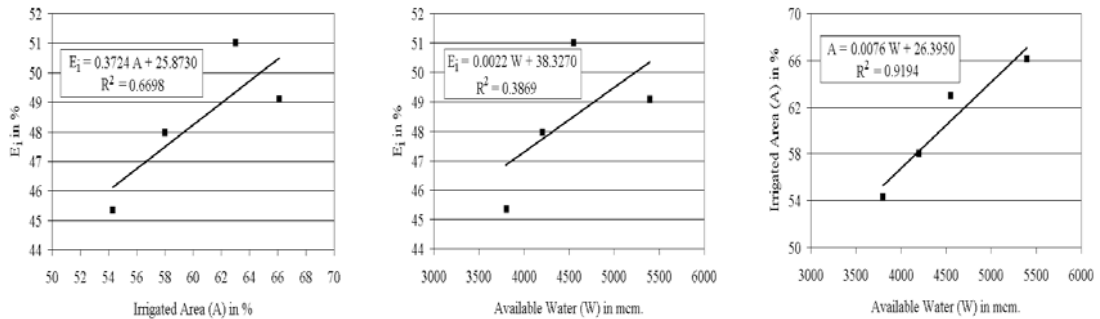
Correlation Coefficients

	Irrigation efficiency (Ei) in%	Available water (W) in mcm.	Irrigated area (A) in%
Irrigation Efficiency(Ei) in%	1		
Available Water(W) in mcm.	0.62	1	
Irrigated Area(A) in%	0.82	.96	1

Figure 8 Effect of irrigated area and the amount of water supply at Mae Klong diversion dam on dry season Ei in GMKP.

bank showed the highest Ei of 66.8% while Thamaka project on the right bank had the lowest Ei of 19.2%. The analysis showed that Ei of each subproject on GCPP and GMKP varied considerably. The wet season Ei on both GCPP and GMKP had a linear relationship with the annual rainfall while the dry season Ei was linearly related to the water available at the beginning of the dry season and the irrigated area. The irrigated area also was highly correlated to the available water.

The ways to improve Ei on GCPP and GMKP were (1) to develop a practical water allocation strategy to increase the effective use of rainfall in wet season and (2) to control or zone the irrigation area in dry season to reduce the water losses.

LITERATURE CITED

Asian Institute of Technology.1994. Study of Potential Development of Water Resources in the Mae Klong River Basin, Vol.II-Main Report. The Office of National Economic and Social Development Board. Bangkok, Thailand. 237p.

Kanoksing,P., V. Vudhivanich, and P. Unjuck-chun.2001. Evaluation of the Overnight Storage Reservoir Utilization for Sugarcane Cultivation. Kasetsart J.(Nat.Sci.) 35 : 23-33.

Kirdpitak, C.1985. Water Management in Thailand. Department of Water Resources Engineering, Kasetsart University, Bangkok. 339p.

Kobayashi, T., S. Promnaret, N. Usuki, and K.Horii. 1994. A model mater management information network system in the central plain of Thailand, pp. 56-67. *In* Irrigation Performance Evaluation for Sustainable Agricultural Development. RAPA FAO. Bangkok.

Vudhivanich,V., J. Kaewkulaya, P. Sopaphun, W. Suidee, and P. Sopsathien.2000. Development of water allocation strategey to increase water use efficiency of irrigation project. Kasetsart J.(Nat.Sci.) 34 : 145-158.

ใบสมัครสมาชิกวิทยาสารเกษตรศาสตร์

ชื่อผู้สมัคร ข้าพเจ้า นาย/นาง/นางสาว...

☐ ขอสมัคร ☐ ขอต่ออายุสมาชิกวิทยาสารเกษตรศาสตร์ เลขที่...

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โปรดแนบสำเนาบัตรประจำตัวหรือจดหมายรับรองของอาจารย์ที่ปรึกษาดังรายละเอียดด้านหลัง)

ใบเสร็จรับเงิน โปรดกรอกใบเสร็จรับเงินในนาม ☐ ข้าพเจ้า ☐ นิติบุคคลชื่อ...

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โทรศัพท์...

ลงชื่อ...ผู้สมัคร

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