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Table 5. Sweetpotato cultivars released by MARDI.

Cultivar	Year of release	Special characteristics
Gendut	1994	Adapted to marginal soils; highly palatable when boiled, steamed or baked; excellent table variety; less sweet and able to substitute Irish potato in some food preparations
Telong	2000	Adapted to marginal soils; suitable for processing into flour
Jalomas	2000	Adapted to marginal soils; suitable for processing into flour; orangey flesh contains carotenoids
VitAto	2007	Contains high ß-carotene; adapted to marginal soils, especially to *bris*; good table variety and also suitable for making a range of food products

now used for bakery products. For example, the level of substitution when using sweetpotato flour is as follows: 100% for cakes; 60% for muffins; and 50% for cookies or biscuits. Sweet buns can be made directly from sweetpotato and can substitute 50% of wheat flour in the original recipe.

Other products which can be made from sweetpotato include premix flours for traditional *kuih* or Malay cakes such as *cek mek molek*, *onde-onde*, *bingka* and *keria* (sweetpotato doughnut). The flour can also be used to make extruded snacks as well as breakfast 'cereal' (somewhat like rice crispies) which is served with milk.

Sweetpotato can also be made into fries, traditionally prepared from imported Irish potato. Fries are sold in fast-food restaurants and as frozen fries in supermarkets for home consumption. They are a favourite among Malaysian youth. It has been estimated that about half the imported Irish potato (valued at RM60 million per year) are made into fries. Other products made directly from sweetpotato are nuggets and breaded sweetpotato. Meat or vegetable pies and cheese bakes are yet other ways of replacing Irish potato with sweetpotato.

PRODUCTION PROSPECTS, CHALLENGES AND FUTURE OPPORTUNITIES

The total sweetpotato area in Malaysia was reported as 1842 ha in 2006, with the largest areas in Selangor (617 ha), Johor (472 ha) and Kelantan (423 ha) [17]. This production caters mainly for the fresh food market, with a small amount of processing into traditional *kuih*, *kerepek* or oil-fried crisps and *cakar ayam*, another traditional snack. Current demand for fresh sweetpotato is inelastic, and any sudden increase in production can lead quickly to depressed prices.

Prospects for expanding production are bright only if demand increases: either as a result of consumers eating more sweetpotato in the traditional ways, or if there is a move towards downstream processing into value-added food products. As mentioned above, there are many marginal soils currently under-utilized which can be used for sweetpotato cultivation. In particular, sweetpotato can become a viable alternative to tobacco-growing on *bris* soils, providing a solution to the less productive tobacco farmers when AFTA comes into force in 2010.

The main challenges facing expanded sweetpotato production are limited current demand (that can be overcome with processing which greatly widens the scope of utilization), and the need for crop rotation. Two major constraints in production are the sweetpotato weevil (*Cylas formicarius* F.) and the sweetpotato virus disease (SPVD) which can build up to unmanageable levels with monocropping. Sweetpotato can be rotated with other short-term crops such as sweetcorn, watermelon, pumpkin, tobacco and yambean (*Pachyrhizus erosus* (L.) Urban). While this is a good agronomic practice, larger scale production for processing will face the challenge of managing a two-crop system.

The prospects for processing sweetpotato into wholesome food products are also good with the availability of the necessary technologies. This has

Table 6. Suggested ways of replacing rice and wheat intake with sweetpotato.*

Breakfast	Lunch/Dinner	Snacks
Sweet buns	Fries	Cakes
Muffins	Boiled sweetpotato	Muffins
Extruded breakfast "cereal"	Steamed sweetpotato	Biscuits
Traditional *kuih* from premixes	Meat/vegetable pies	Extruded snacks
	Cheesy bakes	Traditional *kuih* from premixes
	Nuggets	Vacuum-fried *kerepek*
	Breaded sweetpotato	

*Technologies for all products mentioned area available at MARDI

attracted the interest of a few state government agencies in Terengganu, Kelantan and Kedah, as well as a well-known multi-national food manufacturer which has signed a memorandum of understanding with MARDI to use VitAto cultivar as an ingredient in their processing. The planting of VitAto cultivar is scheduled to expand by 200 ha this year, and very likely by up to 2000 ha subsequently when processing factories are set up as well as when small and medium-sized food manufacturers increase their use of sweetpotato in their products.

CONCLUDING REMARKS

Malaysia can become self-sufficient in rice if several measures are taken:

1. Current rice production areas are expanded;
2. The per capita consumption of rice is reduced. Several diets (including the Atkin's diet) have shown that reducing daily carbohydrate intake can bring about health benefits; and
3. Sweetpotato is used as a supplementary staple. It is an ideal alternative because of its adaptability to local conditions and to marginal soils, as well as the many ways by which it can be eaten (Table 6).

If we replace the rice eaten at lunch and at dinner with sweetpotato just once a week, this will cut down on rice demand by 14%, bringing the current self-sufficiency level to 84%. By eating sweetpotato instead of rice twice a week, Malaysia will reach 98% self-sufficiency at current rice production levels.

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Dragonflies (Insecta: Odonata) from the Maliau Basin, Sabah, Malaysia

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Abstract A survey of Odonata was conducted during the Maliau Basin Scientific Expedition from 18-23 April 2006. A total of 15 species, belonging to nine families was collected. The majority (54.5%) of the 55 specimens collected were from the families Amphipterygidae, Libellulidae and Coenagrionidae. Six of the species are endemic to Borneo: *Devadatta podolestoides*, *Vestalis amnicola*, *Prodasineura hyperythra*, *Coeliccia nemoricola*, *Coeliccia* sp. and *Heliogomphus borneensis*. *Heliogomphus borneensis* had not previously been recorded from Sabah. An annotated list of Odonata collected is provided. This list is by no means exhaustive as it is based upon a limited collecting period; many further species can certainly be expected from the Maliau Basin.

Keywords Odonata – dragonflies – Maliau Basin – Sabah – Borneo – Malaysia

INTRODUCTION

The Odonata, commonly known as dragonflies, consist of two sudorders: the Anisoptera (true dragonflies) and Zygoptera (damselflies). Adult dragonflies are of great attraction to the general public because of the beautiful coloration and fascinating behaviour of many species. They are predators of various insects including disease vector mosquito species and other pests [1-3]. Adult Odonata hunt their prey on the wing, catching it in their legs. Odonata larvae are aquatic; they capture their prey by means of a uniquely modified hinged labium that can be shot forward to grab their prey [2, 4-6].

In tropical Asia, Odonata breed in all freshwater habitats, including lakes, swamp forest, streams and seepages in all forest formations. The highest species diversity of odonates in Sundaland occurs in lowland mixed dipterocarp forest, in particular at pristine streams [2]; swamp forest habitats sometimes also have high odonate diversity. Over 340 species of dragonfly have been recorded from Malaysia including Peninsular Malaysia, Sabah and Sarawak [2, 5; Dow, unpublished]. The figure of 275 named species of Odonata from Borneo [5] is now out-of-date; over 230 species are currently known from Sarawak alone (Dow, unpublished), the same number that is known from Peninsular Malaysia. Many of the species found in Borneo are endemic to the island, comprising about 65% of Zygoptera and 20% of Anisoptera endemic (based on data from [5]). Only 131 species are known from both Peninsular Malaysia and Borneo.

This paper presents the results of a short expedition to the newly established Eucalyptus Camp in the Maliau Basin, located in the southern part of Sabah. An annotated checklist of Odonata so far recorded at the Eucalyptus Camp is provided.

MATERIALS AND METHODS

Study Site

The Maliau Basin is sometimes referred to as 'The Lost World of Sabah', referring to its isolated location and limited accessibility. The formation of the Maliau Basin occurred in the early to middle Miocene age [7]. The basin is formed from a combination of mudstones integrated with layers of siltstone and sandstones [8]. Two forest types occur in the basin – lower montane hill forest and submontane heath forest. A survey of the heath forest in a ca 2 km radius around the Malaiu Basin Field Station indicated that the forest was dominated by the tree families Dipterocarpaceae, Casuarinaceae and Araucariaceae [9]. Parts of the Malaiu Basin have been logged under the Sabah Foundation Logging Concession since 1970.

The Eucalyptus camp is situated at N 04^{0}5 2 ' 18", E 116^{0}49' 35", and is approximately northwest of the Maliau Basin Conservation Area (MBCA). This area has an altitude from ca 950 m to ca 1000 m above sea level. The camp is surrounded by lower montane hill forest and heath forest.

Sampling

Adult Odonata were collected along a stream situated beside the base camp and at several shallow ponds at a helipad about 50 m away from the base camp (see Figure 1) on 18-23 April 2006. Sampling was conducted using hand held nets. Specimens were taken to the Centre for Insect Systematics at Universiti Kebangsaan Malaysia (UKM) for mounting and preserving. Additional specimens were observed and/or photographed in the field but not collected. Specimens were identified to species under a stereomicroscope, by reference to the relevant literature.

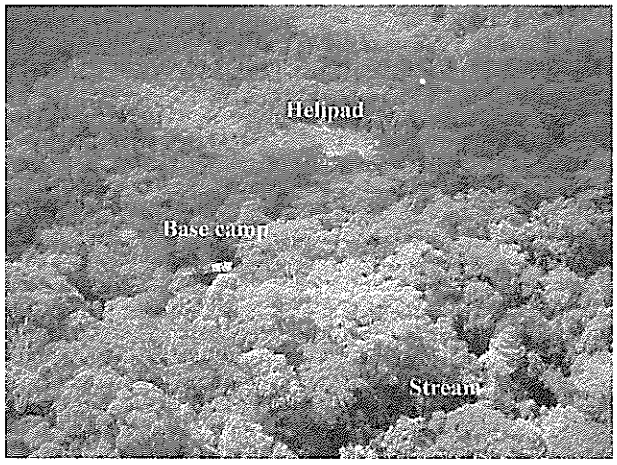

Figure 1. The location of Odonata sampling sites in Maliau Basin, Sabah, Malaysia. (photo: Y.F. Ng)

RESULTS

A total of 55 specimens of adult Odonata were collected, belonging to 9 families. The majority of specimens were from the families Amphipterygidae, Coenagrionidae and Libellulidae. The species collected, and number of specimens, are listed below, along with brief notes.

Zygoptera

Amphipterygidae

1. *Devadatta podolestoides* Laidlaw, 1934 – Seven specimens. A common species at hill and mountain forest streams in North Borneo. Endemic to Borneo.

Euphaeidae

2. *Euphaea impar* (Selys, 1859) – Five specimens. A species of forest streams, more common in lowland areas than at the altitude of Eucalyptus Camp.Calopterygidae
3. *Vestalis amnicola* Lieftinck, 1965 – Five specimens (Fig. 2). Usually found at forest streams in steep terrain, widely distributed in Sabah and Sarawak, but quiet local in occurancu.Endemic to Borneo.

Protoneuridae

4. *Prodasineura hyperythra* (Selys, 1886) – Four specimens. An inconspicuous forest stream dweller. Endemic to Borneo.
5. *Prodasineura verticalis* (Selys, 1860) – Five specimens. A common species of forest streams.

Coenagrionidae

6. *Xiphiagrion cyanomelas* (Selys, 1876) – Nine specimens (Fig. 3). A widespread but local spe-

cies of ponds and lakes. Specimens from Eucalyptus Camp differ from the typical lowland form found in Malaysian Borneo in size and markings, and are similar to specimens from higher altitudes in Java [10] and some material from the Lesser Sunda Islands [11].

Platycnemididae

7. *Coeliccia nemoricola* Laidlaw, 1912 – Three specimens. Only females of this forest stream species were collected. Identification of species from this genus in Borneo is difficult at present, but these specimens agree extremely well with near topotypical material from the Kelabit Highlands of north east Sarawak. Endemic to Borneo.
8. *Coeliccia* sp. – One specimen. A female that appears to differ from any described species in the structure of the prothorax. This might be a species new to science, but determination of its true status awaits the discovery of the male. All species of *Coeliccia* known from Borneo are endemic to the island. If this species is new to science, it may be endemic to the Maliau Basin.

Anisoptera

Gomphidae

9. *Heliogomphus borneensis* Lieftinck, 1964 – One specimen (male). A very poorly known species of forest streams; this is the first record that we are aware of from Sabah. Previously only known from the type series from the Kutai area of east Kalimantan [12] and two males collected at Gunung Mulu National Park in Sarawak, in 2005 [13]. The present record extends not only the known geographical range of the species, but also its altitudinal range; all previous records are from lowland sites. Endemic to Borneo.

Aeshnidae

10. *Indaeschna grubaueri* (Förster, 1904) – One specimen (Fig. 4). A very large species, quite common at forest pools from the lowlands to over 1100 m.

Libellulidae

11. *Agrionoptera sexlineata* Selys, 1879 – One specimen (Fig. 5). A local forest species, most common in swamp forest.

Figure2. *Vestalis amnicola.* (photo:H.S.Yong)

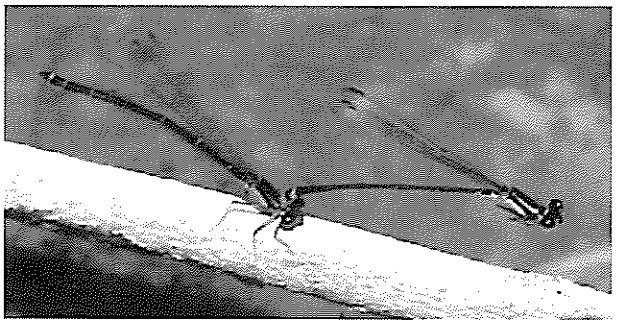

Figure 3. Mating pair of *Xiphiagrion cryanomelas.* (photo: H.S. Yong)

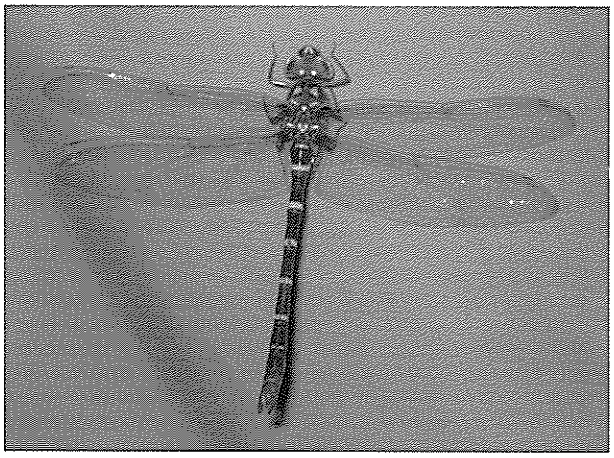

Figure 4. *Indaeschna grubaueri* attracted to light in the camp. (photo: H.S. Yong)

12. *Cratilla metallica* (Brauer, 1878) – One specimen (Fig. 6). A common forest species, found in the same situations as *Indaeschna grubaueri*.
13. *Orthetrum chrysis* (Selys, 1891) – Nine specimens (Fig. 7). A common species at ponds, sometimes also found at forest streams.
14. *Orthetrum pruinosum schneideri* Förster, 1903 – Two specimens (Fig 8). Less common than *O. chrysis*, and usually found at forest pools and slow flowing forest streams.
15. *Pantala flavescens* (Fabricius, 1798) – One specimen. The most widespread odonate in the world, recorded from all continents except for Antarctica.

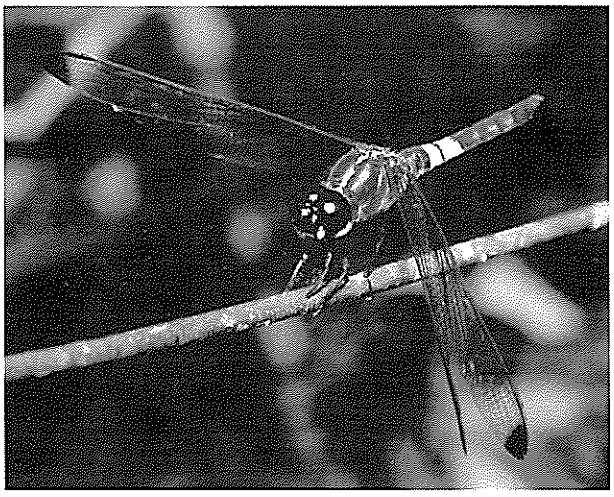

Figure 6. *Cratilla metallica.* (photo: H.S. Yong)

DISCUSSION

A total of 15 species of Odonata was recorded from the vicinity of Eucalyptus Camp. The number of species collected during the expedition is considered low compared to other collections from lowland forests and peat swamp forests. This is due to restrictive conditions such as limited collecting time and adverse weather conditions. For example, at the University of Malaya campus in Kuala Lumpur, over 80 species (constituting some one-third of the

Figure 7. *Orthethrum chrysis.* (photo: H.S. Yong)

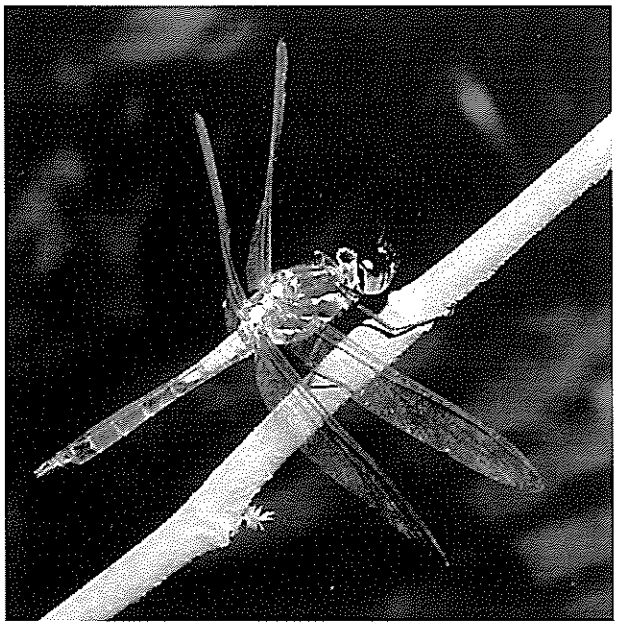

Figure 5. *Agrionoptera sexlineata.* (photo: H.S. Yong)

Figure 8. *Orthethrum pruinosum.* (photo: H.S. Yong)

odonate fauna in Peninsular Malaysia) have been documented over several years [14]. During October 2001, 20 species of odonates were collected at Lake Linumunsut in northern Maliau by Chey Vun Khen and Darline Lim-Hasegawa (Chey Vun Khen, pers. comm.).

The Libellulidae were the most species rich family with five species collected; the Libellulidae are the largest family of the Odonata, and also conspicuous and generally easily collected, so this result was to be expected. A similar pattern is shown in most surveys of Odonata in Malaysia [15]. However, more species (eight) of Zygoptera were collected than of Anisoptera (seven), from six families, compared with three families of Anisoptera. The Protoneuridae and Platycnemididae were each represented by two species; both of these families are well represented in north Borneo. The most significant record made at Eucalyptus Camp to date is that of the poorly known gomphid *Heliogomphus borneensis*. The unidentified and possibly undescribed *Coeliccia* female is also a significant record.

The Odonata of Borneo exhibit relatively high species endemicity, with ca 40% of the species known from the island not found elsewhere. Six species recorded from Eucalyptus Camp are endemic to Borneo; of these one (*Coeliccia* species) might possibly be endemic to the Maliau Basin. As noted above, *Xiphiagrion cyanomelas* from the Maliau Basin differ from examples from the lowlands of Borneo; the form found at Eucalyptus Camp has not been recorded from Borneo before.

Additional families and species are highly likely to be found in the vicinity of Eucalyptus Camp with further sampling. From the Zygoptera, the Chlorocyphidae and the Platystictidae include species likely to be found in the habitats present and at the altitude at the site. From the Anisoptera, members of the Corduliidae can be expected at stream locations; seven species from this family have been recorded at comparable altitudes in the Kelabit Highlands of north east Sarawak [16]. Additional species from other families are also likely to be present. The Maliau Basin is potentially an extremely interesting area for Odonata in north Borneo; further, extensive sampling is urgently needed in this area.

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Comparison of the efficiency of methods and selective agars for enumerating *Vibrio parahaemolyticus* in shrimps

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Abstract *Vibrio parahaemolyticus* is a major food borne pathogen that causes rejection of tiger shrimps (*Penaeus monodon*) consignments at the export market. It was artificially inoculated into tiger shrimps and recovery was evaluated by using nine standard procedures incorporating most probable number (MPN) method or direct plating and four selective agars. The enrichment step in alkaline salt peptone water (ASPW) and 20 h direct plating on thiosulfate citrate bile salts sucrose (TCBS) agar gave the highest recovery (76.9%) of *V. parahaemolyticus* in shrimps and followed by primary and secondary enrichment steps in alkaline peptone water (APW) using MPN method (71.5%). *V. parahaemolyticus* was not recovered in salt polymyxin broth (SPB) using either direct plating or MPN. Three tube MPN method using enrichment media ASPW and APW gave recoveries of 64.2% and 62.1% respectively. The enrichment in glucose salt teepol broth (GSTB) using MPN method resulted in 57.9% recovery whereas it was 24.7% in saline glucose sodium dodecyl sulphate peptone water (GST). The recovery in GST using direct plating was 50.4%. ASPW and APW with double enrichment steps are recommended as the most superior enrichments for enumeration of *V. parahaemolyticus* in tiger shrimps using direct plating and MPN method respectively. The differences observed among four selective media namely TCBS, triphenyl tetrazolium soya tryptone agar (TSAT), sodium dodecyl sulphate polymyxin sucrose agar (SDS) and CHROM agar Vibrio (CV) for enumerating *V. parahaemolyticus* were not significant ($p>0.05$).

Keywords Efficiency – enrichment – enumeration – *Vibrio parahaemolyticus* – shrimps

INTRODUCTION

Vibrio parahaemolyticus is a major food borne pathogen that causes rejection of consignments of tiger shrimps at the export market. Detection and enumeration of *V. parahaemolyticus* in shrimps has become important as some European Union countries have imposed zero tolerance for this organism in their importation of shrimps. In the last three and half years, 39 shrimp consignments produced in Malaysia were rejected in the countries of the European Union due to the presence of *V. parahaemolyticus* (personal communication, Ministry of Health Malaysia). To establish effective control measures to reduce the risk of prevalence of *V. parahaemolyticus* and to ensure the safety of shrimps, efficient analytical methods for the detection of *V. parahaemolyticus* in shrimps and the environment must be available.

There are various methods available for the detection of *V. parahaemolyticus* in food. Quantitative procedures either direct plating or most probable number (MPN) are occasionally used for the enumeration of *V. parahaemolyticus* [1]. It has

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2 Beveridge W.I.B. (1961) *The Art of Scientific Investigation*. Mercury Book, London.

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been noted that different methods to detect *V. parahaemolyticus* have different sensitivities [2]. Through observations and visits in various shrimp factories in Malaysia, it has been noticed that the laboratories for testing of this organism are using different methods and media. Therefore it is high time to compare these methods to determine the most reproducible method for testing of *V. parahaemolyticus*.

There are different methods using different enrichments such as alkaline peptone water (APW), alkaline salt peptone water (ASPW), salt polymyxin broth (SPB), glucose salt teepol broth (GSTB) etc for selective isolation of *V. parahaemolyticus*. Different culture media are also being used for the enumeration of *V. parahaemolyticus*. Thiosulphate citrate bile salts sucrose (TCBS) agar is a selective medium commonly used for the isolation of this organism and other members of the genus *Vibrio* from seafood. This medium supports good growth of most species while inhibiting most non-vibrios [3].

Vibrio mimicus and *Vibrio vulnificus* cannot easily be distinguished from *V. parahaemolyticus* on TCBS agar as they form similar type of green colonies on TCBS [4]. Therefore three other culture media namely: Triphenyl tetrazolium soya tryptone agar (TSAT), Sodium dodecyl sulphate polymyxin sucrose agar (SDS) and CHROM agar Vibrio (CV) that can be used for enumerating *V. parahaemolyticus* were compared to find out the efficiency in recovering un-stressed cells of *V. parahaemolyticus* from artificially contaminated shrimps.

The aim of this study was to find the most reproducible method and culture media for quantitative detection of *V. parahaemolyticus* in shrimps and it was evaluated by comparing nine different standard methods, direct plating or MPN and four selective agars.

MATERIALS AND METHODS

Brackish water shrimps (*Penaeus monodon*) which are commonly known as black tiger shrimps were used in this study. Shrimps needed for the experiments were collected in sterile polythene bags from a shrimp processing plant in Kuala Selangor and transported to the microbiological laboratory at Universiti Kebangsaan Malaysia in an iced styrofoam box and kept in frozen storage (below -18°C) until use. Frozen shrimps were kept in refrigerator (4-8°C) for thawing a few hours before using for the experiment. The muscle portion of shrimp was cut aseptically into small pieces using sterile scalpel. 25 g or 50 g was weighed into a sterile stomacher bag and blended in a stomacher with 225 mL or 450 mL diluent described below under each method at low speed for 30 seconds.

Preparation of inoculum

Pure culture of *V. parahaemolyticus* (clinical O3:K6 strain which was tdh positive and trh negative) confirmed with polymerase chain reaction (PCR) and BIOLOG microlog system was used throughout the study. A loopful of *V. parahaemolyticus* which was stored in long term preservation medium at room temperature was streaked on nutrient agar (3%NA) containing 3% sodium chloride (NaCl) and incubated at 37°C for 18 h. A loopful from this agar was inoculated into brain heart infusion broth containing 3% NaCl and incubated for 18 h at 37°C. The culture was diluted decimally to the inoculum level with sterile 0.1% peptone containing 3% NaCl (pH 7.0) and the level of inoculum was determined by spread plating 0.1 mL portions of dilution on duplicate plates of TCBS agar.

Comparison of methods

The method for detection of *V. parahaemolyticus* by the International Standard Organization (ISO) recommends selective enrichment in SPB and ASPW or saline glucose sodium dodecyl sulphate peptone water (GST). The pH was adjusted at 7.4 in SPB and basal medium was sterilized by autoclaving. Polymyxin B sulphate (PB, CalBiochem) was prepared separately and sterilized by filtration. PB was added to the basal medium before used. 25 g of shrimps were stomached with 225 mL SPB, ASPW (pH 8.6) and GST (pH 8.6) separately and a known concentration (10^4 to 10^5

cfu/mL) of *V. parahaemolyticus* was inoculated. Decimal dilutions (from 10^{-2} to 10^{-6}) were prepared in 0.1% peptone containing 3% NaCl (pH 7.0). Dilutions from 10^{-1} to 10^{-4} were spread plated (0.1 mL) on TCBS (Oxoid) agar for direct enumeration. One mL from each dilution (from 10^{-1} to 10^{-6}) was transferred to 10 mL of SPB, ASPW and GST separately in 3-tube MPN method. Tubes and plates were incubated at 37°C for 20 h. Tubes with visible growth were streaked on TCBS and TSAT agar and incubated at 37°C for 20 h. Results were calculated in MPN/g and cfu/g based on number of tubes that yielded growth of organism on TCBS and TSAT and colonies on plates of TCBS agar [5].

The procedure for testing of *V. parahaemolyticus* recommended by the Ministry of Health (MOH) Malaysia is based on the Australian/New Zealand Standard. 25 g of shrimps was stomached with 225 mL of 0.1% peptone with 3% NaCl and a known concentration of *V. parahaemolyticus* was inoculated. Decimal dilutions were prepared in 0.1% peptone (3% NaCl). One mL from each dilution was transferred to 10 mL of APW (primary enrichment, pH 8.6). Tubes were incubated at 37°C for 6 h and 1 mL from each tube was transferred to freshly prepared tubes of 10 mL APW (secondary enrichment). A loopful from each primary enrichment medium was streaked on TCBS agar. Secondary enrichments and plates were incubated at 37°C for 18 h. A loopful from each secondary enrichment medium was streaked on TCBS agar and incubated at 37°C for 18 h. The tubes from which *V. parahaemolyticus* cells were detected on plates were considered to be positive for this organism. Results were calculated in MPN/g [6].

The American Public Health Association (APHA) describes the method as follows: 50 g of shrimps was stomached with 450 mL of phosphate buffered saline (PBS, pH 7.2) and inoculated with *V. parahaemolyticus*. Dilutions were prepared (from 10^{-2} to 10^{-6}) in PBS and 10 mL from 1:10 homogenate was added into 3 tubes of double strength APW (10ml). Similarly 1 mL from all dilutions (from 10^{-1} to 10^{-6}) was inoculated into 10 mL of single strength APW and incubated at 37°C for overnight. A loopful from APW showing growth was streaked on TCBS agar and incubated at 37°C for overnight. The plates were examined for *V. parahaemolyticus* colonies, and the MPN values were calculated [7].

According to the method described by the Food and Agriculture Organization (FAO), 450 mL of 3% dilution water was added into 50 g of shrimps and blended. *V. parahaemolyticus* was inoculated and decimal dilutions were prepared. Ten mL from 1:10 dilution was added into 3 tubes of double strength GSTB (10ml, pH 7.4). One mL from all other dilutions was transferred into 10 mL of single strength (3 tubes) GSTB (pH 7.4). After overnight incubation at 37°C tubes showing growth were streaked on TCBS agar and incubated at 37°C. The plates were examined for *V. parahaemolyticus* colonies, and the MPN values were calculated [8].

Comparison of culture media

25 g of shrimps was stomached with 225 mL of 0.1% peptone containing 3% NaCl. Inoculum of *V. parahaemolyticus* was prepared as mentioned above and serial ten fold dilutions in 0.1% peptone (3%) were prepared. Shrimps were artificially inoculated with a final estimated *V. parahaemolyticus* concentration of 10^5-10^6 cfu/g. Decimal dilutions (10^{-2}-10^4) were prepared in 0.1% peptone (3%) and mixed well by vortex. Each dilution (from 10^1-10^4) was spread plated (0.1 mL) on TCBS agar, TSAT agar, SDS agar and CV agar in duplicate for direct enumeration. Plates were then incubated at 37°C for 20 h and colonies of *V. parahaemolyticus* were counted in cfu/g.

Recovered organisms in shrimps were confirmed as *V. parahaemolyticus* using Analytical Profile Index (API 20E, bioMerieux) and minimal number of biochemical tests [7] in each method. API 20E identification was done according to the manufacture's instructions.

Un-inoculated samples of shrimp were tested in parallel using each method to confirm the absence of naturally occurring *V. parahaemolyticus* in shrimps used for the experiment. Percentage re-

covery of *V. parahaemolyticus* was calculated based on the approximate level of inoculum (cfu/mL) in each occasion and the number of organisms recovered in each method. All experiments were repeated usually three times and in each case three samples (triplicates) were analyzed independently.

Statistical analysis

In the first experiment, the number of organisms recovered was calculated as a percentage and in the second experiment the number of organisms recovered was converted to $\log_{10}$ cfu/g and analyzed statistically by ANOVA using the SPSS 13.1. Means of three replicates were reported. Differences among methods of testing and culture media were examined for level of significance ($p<0.05$) by Duncan's multiple range test.

RESULTS AND DISCUSSION

Data comparing methods of testing for *V. parahaemolyticus* by quantitative recoveries are presented in Figure 1. The performances of 20 h direct plating method in ASPW were superior to that of other methods and gave the highest recovery of 76.9%. The next highest recovery of 71.5% was obtained from primary and secondary enrichments

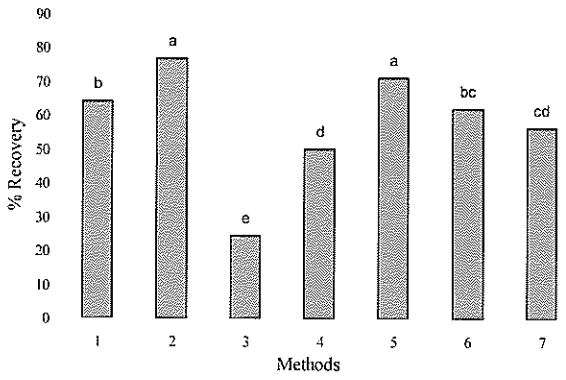

Figure 1. Percentage recovery of *V. parahaemolyticus* in shrimps (*Penaeus monodon*) using different methods. 1: ISO-ASPW MPN; 2: ISO-ASPW Direct plating; 3: ISO-GST MPN; 4: ISO-GST Direct plating; 5: AS/NZS-APW MPN; 6: APHA-APW MPN; 7: FAO- GSTB MPN. a-e: Different alphabets for different methods show significant differences ($P<0.05$). Each bar represents average of three independent experiments.

in APW using MPN method. The difference observed was not significant among these two methods. Two steps enrichment in APW using MPN method resulted in significantly higher recovery (71.5%) than any single step enrichment MPN method described in this text. It was noticed that *V. parahaemolyticus* colonies were well isolated in this method than any other methods described here.

Vibrio parahaemolyticus was not recovered in SPB using either direct plating or MPN. Concentration of PB in SPB was 12.34 µg/mL or 100 IU/mL taking 1 mg as equivalent to 8100 IU, as with PB according to manufacturer's instructions. Ottaviani *et al.* [9] also found that growth was inhibited in SPB for many microorganisms. Kampelmacher *et al.* [10] found that some strains of *V. parahaemolyticus* in pure culture appear to be inhibited by 70 IU per mL of PB. The unsuitability of SPB with 250 µg of polymyxin per mL was indicated by studies of Karunasagar *et al.* [11] on 27 samples of raw and processed shrimp, of which 10 samples showed the presence of *V. parahaemolyticus* when GSTB was the enrichment broth, whereas none were positive when SPB (250 µg of polymyxin per mL) was used. They compared recovery of *V. parahaemolyticus* from inoculated fish homogenates using various enrichment broths namely: salt broth, SPB (0.25µg/mL and 2.5µg/mL of Polymyxin), GSTB and direct plating on TCBS. They concluded that recovery by direct plating appeared to be good. This suggests that during the period of incubation of homogenates in broths, the *V. parahaemolyticus* isolates are overgrown by the other flora. This was further supported by the observation from sterile fish homogenates inoculated with *V. parahaemolyticus* cells reached the upper limit of detection by the MPN technique in salt broth and SPB with 0.25 µg of polymyxin per mL. Even in the absence of competing flora, SPB with 2.5 µg of polymyxin per mL did not yield good recovery, suggesting the toxicity of polymyxin to *V. parahaemolyticus*. The least recovery was observed in GSTB. Based on these results Karunasagar *et al.* [11] suggested that direct plating on TCBS might be more useful in obtaining a good estimate of

the *V. parahaemolyticus* counts in seafood. This technique is less laborious and results are obtained a day earlier as compared with the MPN technique. Their findings are also in agreement with our present findings. Hagen *et al.* [12] compared two enrichment broths (APW and SPB) for their ability to recover *V. parahaemolyticus* inoculated into each of five seafoods (crab legs, oysters, shrimps, lobsters and sharks). Recovery of *V. parahaemolyticus* from each seafood immediately after inoculation was significantly higher ($P<0.05$) with APW than with SPB. The concentration of PB in SPB was 2.5µg/mL and pH 7.4.

Three tube MPN method using enrichment media such as ASPW (ISO) and APW (APHA) gave recoveries of 64.2% and 62.1% respectively and there was no significant difference between these two methods. Eyles *et al.* [13] and Oscroft [14] also found that APW was most effective for the isolation of *V. parahaemolyticus* from oysters and prawns. The enrichment in GSTB using MPN method resulted in 56.7% recovery whereas it was 24.7% in GST. The recovery in GST using direct plating was 50.4%.

Figure 2 represents the recovery of *V. parahaemolyticus* in log cfu/g using four plating media. The differences observed among four media were not significant ($p=0.932$). Colonies of *V. parahaemolyticus* observed on TCBS was green in colour and the size was 2-5 mm. Selective agents in TCBS are sodium thiosulphate, sodium taurocholate, ferric citrate and pH of 8.6 while the diagnostic agent is sucrose [15]. *V. parahaemolyticus* produced dark red colonies with a diameter of more than 2 mm on TSAT. Bile salt is the selective agent in TSAT while sucrose and triphenyltetrazolium chloride (TTC) are diagnostic agents [16]. Green colour and 2-5 mm size colonies were observed on SDS agar. Selective agent in SDS is sodium dodecyl sulphate and the diagnostic agents are sucrose and sulphatase [17]. The colonies produced on CV were violet in colour. CV medium containing substrates for beta-galactosidase was developed specifically to differentiate *V. parahaemolyticus* from other bacteria by using a chromogenic substrate, instead of sugar fermentation, used in traditional growth media such as TCBS [4].

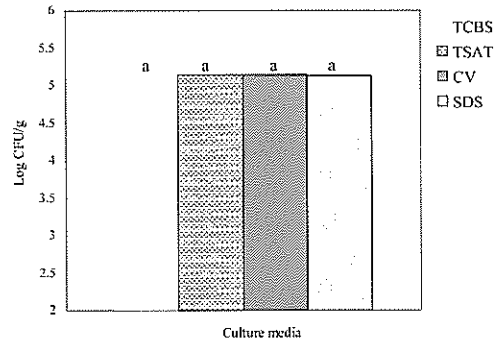

Figure 2. Recovery of *V. parahaemolyticus* on different culture media. a: same alphabet for different culture media indicates no significant differences ($P>0.05$).

There was no any advantage over the other in selective agents or diagnostic agents in four culture media compared for unstressed pure organisms of *V. parahaemolyticus*. It is required to conduct field studies to evaluate the behaviour of background microflora when isolating *V. parahaemolyticus* from culture environment. Estuarine waters generally contain a significant number of *Pseudomonas* species and to a lesser extent *Flavobacterium* and *Photobacterium* species [18]. Thus a medium intended for use in such environments must be able to inhibit or at least differentiate among such genera. It was noted by Villari *et al.* [19] that the choice of the culture medium may depend on the expected level of bacterial contamination of the sample. Samples with an expected high level of background microbial flora should be analyzed through more selective media [19].

In sum, ASPW with single enrichment step is recommended as the most superior method for enumeration of *V. parahaemolyticus* in shrimps using direct plating technique. There was no significant difference in the recovery of *V. parahaemolyticus* on TCBS, TSAT, SDS or CHROM agar *Vibrio*. The plating efficiency of TSAT agar, SDS agar and CHROM agar *Vibrio* appeared to be comparable to that of TCBS, which is the most commonly used media for enumeration of *V. parahaemolyticus*.

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Critical habitats for the survival of Malayan mammals in Peninsular Malaysia

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Abstract The forest habitats below 1000 m in elevation are the most critical for the survival of mammalian species in Peninsular Malaysia. These habitats comprise the lowland and hill dipterocarp forests, peat and freshwater swamp forests and mangrove forests. Of the 229 mammalian species (volant and non-volant species) 61.6% inhabit only lowland/hill forests below 1000 m while 10.9% occur only above 1000 m in elevation. The remaining 27.5% are free ranging from lowland to montane forests. Thus, the lowland/hill forests under 1000 m are of particular significance for the survival of species and species diversity in this country.

The continuous loss of lowland/hill forests to agriculture and other development, if left unchecked, will have disastrous consequences on all large mammal species and other rare and endemic species. These species will be seriously threatened as their numbers decrease with the increasing encroachment and destruction of their habitat. Seven orders (Primates, Insectivora, Scandentia, Perissodactyla, Proboscidea, Pholidota, Dermoptera; those with 10 species or less) out of 11 orders in Peninsular Malaysia would be among the first to disappear. Of particular vulnerability are the animals that inhabit the lowland forest canopy. The progressive destruction of the lowland/hill primary habitat, except for a few isolated blocks of pristine lowland habitats such as Taman Negara, Krau Wildlife Reserve and the Endau Rompin Park (Johor and Pahang), will result in the elimination of many of the native fauna of the forest and its replacement by commensal species. With commensal species, the spread of zoonotic and vector borne diseases to humans will increase, especially where there is poor management and unsanitary conditions are found.

Keywords critical habitats – survival – conservation – Malayan mammals – Peninsular Malaysia

INTRODUCTION

For the purpose of this paper a critical habitat is defined by two factors. First it must be a habitat required for the long term survival of a species (fauna or flora). Second the size (area basis) and condition of this habitat must be able to support a viable population of the species concerned. In Peninsular Malaysia, 229 species of terrestrial mammals comprising 34 families from 11 orders have been described. These comprise 124 non-volant and 105 volant species. The latter are all bats [1, 2]. Based on records from over one hundred years of field studies, an analysis is made on the host-habitat association for the different orders of mammals.

HABITAT PREFERENCE

Research on the classification of habitat preferences by mammalian hosts was initiated by Harrison [3]. Stevens [4] and Yong [5] also contributed towards the effort. This paper reviews the topic and incorporates records and recollections of the author from

over 37 years research at the IMR, the Wildlife Department (currently the Department of Wildlife and National Parks (DWNP)), Forest Research Institute Malaysia (FRIM) and Universiti Kebangsaan Malaysia (UKM). The term 'usual habitat' is used to typify the permanent natural environment upon which each mammal species is dependent. These habitats include primary and secondary forests, scrub (sometimes including remnant patches of forest), and various types of cultivated areas. For the purpose of this paper we are looking at three habitats, viz. primary forest, secondary forest and disturbed habitats (which include all the other highly modified habitats). The term 'sub-habitat' is employed to describe any preferred arena within these habitats where an animal spends a significant part of its time. Examples of sub-habitats include the arboreal, terrestrial or semi-aquatic niches and of some significance in this paper, human habitations. Elevation refers to the range of elevation that the species occupies. This is divided into lowland, hill and mountain elevations the definitions of which are included in this paper.

USUAL HABITAT OF MALAYSIAN MAMMALS

Four broad habitat-types are considered. Primary forest (P) is pristine or nearly pristine natural forests that have not been subjected to large scale, high impact disturbance, such as logging. Secondary forest (S) is regenerating logged over forest where the logging was carried out about 30 or more years ago (this is arbitrary; the author has observed that when a lowland forest was logged in accordance to good management prescriptions (e.g. the MUS), that forest would begin to be good habitat for most primary forest animals around 30 years after logging; a longer period is needed for montane forests and forests subjected to unsupervised and unregulated logging – as the thrust of this paper is directed upon lowland species, 30 years of regeneration is used for our definition). Disturbed secondary forests and cultivated habitats (DS+C) are forests that have been logged less than 30 years ago, cultivated areas and adjoining scrub habitats. Human habitation (HH) refers to areas where human beings live and their surroundings and would include rural, suburban and urban sites.

Of the 229 mammalian species (volant and non-volant mammals), the primary forest habitat-type has the highest species diversity comprising 113 species (49.3%), followed by 85 species (37.17%) in secondary forest, and 24 species (10.5%) that inhabit between disturbed forest and cultivation forest respectively (Table 1). Only 7 species (3.1%) are found residing within human inhabited areas.

Generally, mammals are highly mobile and move between habitats in search of food. It is thus difficult to limit them to within one habitat system.

Table 1. Usual habits of Malaysian mammals (ranked by increasing number of species). P, Primary forest; S, Secondary forest; DS+C, Disturbed secondary + cultivated areas; HH, Human habitation.

Order	Families	Species	P	S	DS+C	HH
Dermoptera	1	1	-	-	1	-
Pholidota	1	1	-	-	1	-
Proboscidea	1	1	1	-	-	-
Perissodactyla	2	2	2	-	-	-
Scandentia	1	3	2	1	-	-
Artiodactyla	3	9	3	6	1	-
Insectivora	3	10	4	3	2	1
Primates	3	10	5	3	2	-
Carnivora	6	32	11	17	4	-
Rodentia	5	55	27	18	6	4
Chiroptera	8	105	58	37	7	3
No. of species	34	229	113	85	24	7
Percentage (%)	-	-	49.3	37.1	10.5	3.1

However, there is little doubt that most of the species require good forest cover as 198 species or 86.5% of the all the species are dependent on primary and secondary forests for their survival.

SUB-HABITATS OF MALAYSIAN MAMMALS

Animal species demonstrate different behavioral patterns, some spending most of their time on trees or on the ground or in the water. Bats for example, are flying mammals and most species roost in caves, others on trees or in tree-holes. However, they roost above ground level and as such are all treated as being arboreal in habit. With the inclusion of the bats, 167 mammal species (72.9%) are arboreal in nature, 57 species (24.9%) are terrestrial while 5 species (2.2%) are aquatic (Table 2). The dependence of the arboreal species on tree canopy is directly linked to their feeding behaviour. They feed principally on leaves, fruits and shoots and sometimes on arboreal insects as well. The terrestrial or ground-dwelling mammal species are mainly rodent species which are also burrowers. All large mammals are strictly terrestrial, while only a few species are semi-aquatic in behaviour and they feed on aquatic prey species.

Table 2. Sub-habitats of Malaysian mammals.A,arboreal; T, terrestrial; AQ, aquatic.

Order	A	T	AQ	Total
Dermoptera	1	-	-	1
Pholidota	-	1	-	1
Proboscidea	-	1	-	1
Perissodactyla	-	2	-	2
Scandentia	2	1	-	3
Artiodactyla	9	-	-	9
Insectivora	-	9	1	10
Primates	10	-	-	10
Carnivora	15	13	4	32
Rodentia	25	30	-	55
Chiroptera	105	-		105
No. of species	167	57	5	229
Percentage (%)	72.9	24.9	2.2	-

ALTITUDINAL DISTRIBUTION OF MALAYSIAN MAMMALS

Some Malaysian mammals are also limited to certain habitats by altitude. This factor applies mainly to the forest habitats. Most of the forest lowland mammals occupy habitats ranging from lowland to hill forests. Some species are restricted to higher elevations from hill to montane forests. In contrast, species which are highly adaptable to changing landscapes (including deforestation) are designated herein as free-ranging, ranging from lowland to hill and mountain elevations [2, 6].

141 species (61.6%) of Malaysian mammals inhabit lowland/hill forest between <1000 m, 25 species (10.9%) are living >1000 m in elevation and 63 species (27.5%) are free-ranging (Table 3). This clearly shows that lowland/hill forest below 1000 m is the prime altitudinal zone for the majority of mammal species.

Table 3. Altitudinal distribution of Malaysian mammals. L-H, Lowland to hill forest <1000 m, UH-M, Upper hill to montane forest >1000 m; FR, Free-ranging from lowland to montane.

Order	L-H <1000 m	UH-M >1000 m	FR	Totalspecies
Dermoptera	1	-	-	1
Pholidota	1	-	-	1
Proboscidea	1	-	-	1
Perissodactyla	1	1	-	2
Scandentia	3	-	-	3
Artiodactyla	8	-	1	9
Insectivora	4	3	3	10
Primates	8	2	-	10
Carnivora	23	-	9	32
Rodentia	27	10	18	55
Chiroptera	64	9	32	105
No. of spp.	141	25	63	229
Percentage (%)	61.6	10.9	27.5	-

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Editorial: Science for Development

At the recent world congress on IT in Kuala Lumpur, Dr. J Craig Venter said "we are now in a world that is 100 % dependent on science in the future". Science indeed played a pivotal role in the development of mankind. The President of the Academy of Sciences Malaysia (ASM) recognized that many varied activities exist in Science and classified them into (a) Advances in Science and (b) Science for development as reflected in applied sciences.

It is universally recognized that developments in fundamental sciences (Biology, Chemistry, Physics and Mathematics) extend the frontiers of scientific knowledge and through such significant developments the world makes advancement to the next level. Applied sciences such as Agricultural sciences and Forestry, Earth sciences, Engineering sciences, Medical and Health sciences and ICT can be considered as science for development and thus such sciences could be harnessed for health improvement among others and thus contribute to the alleviation of poverty. The obvious examples are tele-medicine, which would enable the improvement of health of isolated community; agrobiotechnology which provides opportunities for improvement in agriculture via improvement in productivity; infrastructure engineering which could open up the rural areas thus providing market access to rural communities and many others.

The most current challenge is to ensure adequate supplies of food at affordable prices to the world population. It is hoped that researchers in science and technology will rise to the occasion to meet the millennium development goals set by the United Nations.

Academician Professor Emeritus
Tan Sri Datuk Dr Augustine S. H. Ong
Co-Chairman, Editorial Board

LOCAL DISTRIBUTION OF MALAYSIAN MAMMALS

It is often found that most mammal species of widespread occurrence are also seldom abundant locally. This is particularly true of the large mammals, the smaller carnivores, some primates, most flying squirrel species and some civets and otters. Some species have limited distributions, either geographically or to high elevations. Such species are generally also uncommon. This may include endemic species which are often confined to small and highly specialized habitats and niches and consequently, exceptionally rare.

Of the 229 species, 158 species (70.0%) are widespread, 63 species (27.5%) are restricted in distribution and 8 species (3.5%) are endemic species (Table 4). The majority of these species occupy forest habitats below 1000 m in elevation.

NUMERICAL STATUS OF MALAYSIAN MAMMALS

The numerical status of each of the species as stated herein, such as 'Abundant, Common, or Rare' is based on trapping results from over 60 years of field data.

(a) Abundant denotes that one or more individuals of the species were trapped in a trapping session that utilized 100 traps over 5 nights (500 trap nights) or were sighted opportunistically over that same period.

(b) Common denotes that more than 5 individuals were trapped over 10 trapping sessions (each trapping session of 500 trap nights).

(c) Rare denotes that not more than 5 individuals were trapped over 10 trapping sessions.

Excluding the volant mammals, of the 124 non-volant species 18 species (14.5%) are abundant, 59 species (47.6%) are common and 47 species (37.9%) are rare (Table 5). Nearly all the abundant mammals are commensal species. The common and rare mammals are all forest species with most of them from lowland forest below 1000 m in elevation.

CRITICAL HABITATS

The lowland/hill forest habitats below 1000 m house the majority of the fauna. This habitat includes lowland and hill dipterocarp forests, peat and freshwater swamp forests and mangrove forests. The large carnivores (bears, tigers, leopards), ungulates (tapir, rhinoceros, dear, gaur, serow), primates (gibbons, leaf monkeys, slow loris) and most of the rare and endemic species of small mammals (felids, mustelids, viverrids, sciurids, murids, chiropterids, etc) are resident and dependent on these forest types for their existence and survival.

This clearly shows that species survival and

Table 4. Local distribution of Malaysian mammals. WS, Widespread throughout Peninsular Malaysia; R, Restricted distribution, highland and other spotted localities; E, Endemic species in Peninsular Malaysia.

Order	WS	R	E	Totalspecies
Dermoptera	1	-	-	1
Pholidota	1	-	-	1
Proboscidea	1	-	-	1
Perissodactyla	1	1	-	2
Scandentia	3	-	-	3
Artiodactyla	6	3	-	-
Insectivora	5	3	2	10
Primates	6	4	-	10
Carnivora	24	8	-	32
Rodentia	40	14	1	55
Chiroptera	70	30	5	105
No. of spp.	158	63	8	229
Percentage (%)	70.0	27.5	3.5	-

Table 5. Numerical status of Malaysian mammals. A, Abundant – denotes at least one individual trapped of each species in each trapping session (of 500 trap nights); C, Common – denotes more than 5 individuals were trapped over 10 trapping sessions (each of 500 trap nights); R, Rare denotes that not more than 5 individuals were trapped or sighted over 10 trapping sessions. The numerical status of the Chiropterans cannot be determined reliably.

Order	A	C	R	Total Species
Dermoptera	-	1	-	1
Pholidota	-	1	-	1
Proboscidea	-	1	-	1
Perissodactyla	-	1	1	2
Scandentia	1	1	1	3
Artiodactyla	-	7	2	9
Insectivora	1	5	4	10
Primates	1	6	3	10
Carnivora	1	17	14	32
Rodentia	10	23	22	55
No. of species	18	59	47	124
Percentage (%)	14.5	47.6	37.9	100

diversity is significantly dependent upon the lowland/ hill forest (from sea level to 1000 m). Large areas of these habitats have been converted to agriculture, industrial development and urbanization. The upper hill and montane forests, in comparison, have been less developed and a higher percentage of forests on mountain tops are still intact. As such, survival of the montane residents (11% of the 229 species) is not as acute as those in the lowland and hill forests.

As more lowland and hill areas are likely to be opened up for agricultural and other land development, the number of those species totally dependent on these forest habitats may be expected to fall rapidly and reach a stage where their survival will be in doubt. All large mammal species, rare and endemic species will be seriously threatened as their numbers decrease with the increasing encroachment and destruction of their habitats. The mammalian orders (Dermoptera, Perissodactyla, Pholidota, Proboscidea, Artiodactyla, Scandentia, Primate and Insectivora) with 10 or less species (Table 1), and especially those with restricted and endemic distribution patterns (Table 4) and those which are numerically rare (Table 5) (Dermoptera, Perissodactyla, Pholidota and Proboscidea) would most likely be among the first to disappear, followed by those that are slightly more common. The progressive destruction of the primary lowland and hill forest habitats will result in the progressive elimination of the native fauna of these forests the ecological niches of which will be taken over by commensal species such as the rodents.

The highland forest habitat may also face the same dilemma in the not so distant future should the development of montane areas for agriculture and recreation, remain unchecked and most of the resident fauna will eventually suffer the same fate as those in the lowland and hill forests.

CONSERVATION STATUS

The main threat to the survival of the Malaysian fauna is habitat disturbance and loss. The most critical habitat type is that of the lowland and hill forests, those natural habitats below 1000 m in elevation where the significant appropriation of land and its easy exploitation due to accessibility is of great concern to those who desire to see the long term survival of species of these habitats. The concern for this habitat is related to two factors. First, almost all lowland forests have been converted to agriculture, industry, urban development, road and power systems or mining. In Peninsular Malaysia very little of such habitat is left in a pristine state with the exception of two major blocks being found in the lowlands of Taman Negara and the Krau Wildlife Re-

serve, in addition to a scattering of smaller wildlife reserves. Certain parts of the Forest Estate referred to as Protection Forests may also enter this picture, except that they are mainly located at montane elevations. There are also areas called Virgin Jungle Reserves, but these are small in area and would not contribute as habitat for wild mammals.

Another category of forests is the Production forests. These could play a long term role in the conservation of species if they are managed on a permanent polycyclic harvesting system. However this and the fact that they are the first area to be assigned for development, does not portend well for any role they may play in the conservation of the mammal fauna. The sudden declassification in Selangor of the Sungei Buloh Forest Reserve, Peninsula Malaysia's oldest Forest Reserve, is a stark example of what can happen. Conservationists should pay particular attention to the preservation of the lowlands of Krau and Taman Negara, the two largest blocks remaining in their efforts in preserving both the flora and fauna.

The small mammals have received very little or no attention because the majority of the species have no direct importance or significance to man. Little is realized that each species is a component of the ecosystem in which it lives and the part it plays to keep and maintain the ecological balance both at the micro and macro levels. Their role as pollinators and seed dispersers and biological agents for pest insects have hardly been highlighted, resulting in them being treated as a step child in comparison to the large mammals whose role as biological agents in the natural forest is highly limited due to the small numbers in existence. The latter mammal group is designated a 'Flag-ship species', thus they always receive greater recognition by conservationists and policy makers in terms of funding for biological research.

Conservation activities on the impact of habitat loss and degradation on the larger mammals (rhinoceros, tiger, elephant, gaur) have been well studied by governmental agents and non-governmental organizations in this country since 1960 [7-19]. Although much information on the ecology, population dynamics, host-habitat relationships etc has been published the population of each of these large mammals has continuously been on the decline, probably due to continuing habitat loss and lack of, or ineffective, enforcement against persistent poaching activities.

When the conservation status of the orders of the wild mammal fauna is examined, we find that no order can be considered safe from possible extinction. Among the carnivorous groups, we find that there is no certainty that the long term survival of the tiger can be assured. The same may be said of all the smaller cats with the exception of the leopard cat which appears to have proliferated in oil palm plantations. The same comment may be made of the wild dog (serigala). It is far from demonstrated that the extant populations in Taman Negara and the Krau Wildlife Reserve, constitute long term viable breeding populations of these species. The deterioration of rivers and water bodies also raises doubts as to the long term survival of the different aquatic predators.

The picture for the non-carnivorous mammalian orders is also doubtful. For example, the moonrat may loose its viability if the remnant lowland peat swamp forests are drained, developed and fragmented. At present the only extensive system remaining is on the coastal plain of southern Pahang. Among the civets, only the common palm civet appears to have adapted to human development and can survive in agriculture and around human habitation. For the rest of the civets, question is whether the lowlands of Krau and Taman Negara can support long term viable populations. The diurnal squirrels are locally plentiful and therefore appear to be safe for the future. However, the flying squirrels are relatively rare and numbers may not be sustained within the two lowland areas of Krau and Taman Negara. Two species may be noted here for this concern. They are the Smoky and Black flying squirrels. Little is known of the population density, ecological needs and survival potential of the other small flying squirrels.

Among the bats, our largest species, the flying fox and its smaller cousin the island flying fox,

are so decimated in numbers, that urgent practical measures are needed if they are to survive in the long term. These measures would include reduction in their being poached and preservation of their roosting habitats. With some of the fruit and most of the insectivorous bats, the survival and protection of their roosting habitats needs to be considered.

Unfortunately for most of these species, there is a lack of in-depth information on their population, distribution and ecological requirements to even understand their conservation status.

The small mammals, on the other hand, are either medium-small to tiny in size and comprise the group with the highest species diversity. In comparison to the large mammals they are not spectacular in terms of size or reputation. Most of them such as the shrews, rats, squirrels including some small carnivores, like civets, mongooses, wildcats, bats, are nocturnal in habit and are seldom seen. Thus they are often ignored by conservationists and forest managers as unimportant in the forest ecosystem. However, the indications are that these small and elusive creatures have greater and more significant roles than hitherto suspected. In the forest, some may serve as pollinators and seed dispersers. They can also be pests, vectors of diseases and control agents of pest species besides being prey species to some large mammal predators [20-32].

DEFORESTATION AND HUMAN HEALTH

The progressive deforestation of the primary forest does not only eliminate the prime animal habitats but also create new habitat complexes and conditions that engender zoonoses and vector borne diseases (Fig. 1).

In the natural pristine environment of the tropical rain forest ecosystems, the development and outbreak of zoonoses and vector borne diseases in human society appear to be naturally contained. In fact, these zoonotic agents and diseases are believed to play a role in the natural control of many vertebrate species in the wild. When the natural state of forest ecosystems is maintained, these zoonoses remain dormant (from the human health point of view) and transmission is confined to the natural parasites. The diseases thus remain restricted and endemic within the spatio-temporal assemblage of the forest ecosystem.

When the forest ecosystems are severely modified, such as from severe logging and clearing for conversion to other land use and agriculture, the balance involving the natural vectors, hosts and parasites is upset. Coupled with the invasions of commensals and other vectors, and humans, the potential for outbreak of zoonotic diseases increases significantly, especially when there are unsanitary conditions such as open rubbish dumps, stagnant water and unsanitary behaviour, which results in the establishment of secondary reservoirs of infection.

However, the spread of zoonotic and vector borne diseases can be controlled with good sanitation and well managed agricultural land use. The impacts of disease from severe often unsustainable logging activities can also be controlled through better forest management and harvesting practices such as through the use of appropriate operations and technologies which can significantly minimize the impact on the physical environment. These can include the use of helicopter logging, by far the least damaging timber harvest system and sequential harvesting of non-adjacent blocks so that there are always relatively undisturbed adjacent forest patches to provide shelter and buffer for animals displaced from the area during logging, and migration of host species will be minimized. This will also ensure that adjacent patches of forests are at different stages of regeneration providing a diversity and range of suitable habitats and niches for the host species. This approach will help contain the zoonotic and vector borne diseases.

CONCLUSION AND RECOMMENDATIONS

Based on the result of the analysis of the fauna in Peninsular Malaysia, it is obvious that the forest cover of the lowland/hill dipterocarp forests <1000 m encompass the most critical habitats for the survival of

the Peninsula's mammalian fauna. The large mammals or flagship species have received much emphasis in conservation and management by those concerned with the survival of species including government and non government conservation agencies. The small mammals, in contrast, have been largely neglected due to a lack of understanding of the important indirect part they play in the forest ecology and agricultural productivity. Their roles include being prey species, bioagents of pests, ectoparasites and disease vectors for the larger high profile mammals. These last named activities also include their impact upon human society.

There is a lamentable lack of interest and research on many mammal species at present, in particular the smaller species. Here we are caught in a classical 'chicken and egg' argument. We think that many of these species have no useful role and therefore we do not consider them as targets of study or conservation. Yet we do not know their actual role because we have not studied them sufficiently. This cycle is vicious and as scientist we need to break it. The obvious approach is to begin a new and urgent programme of basic research, augmented where needed by applied research. This forms the thrust of the suggested recommendations below.

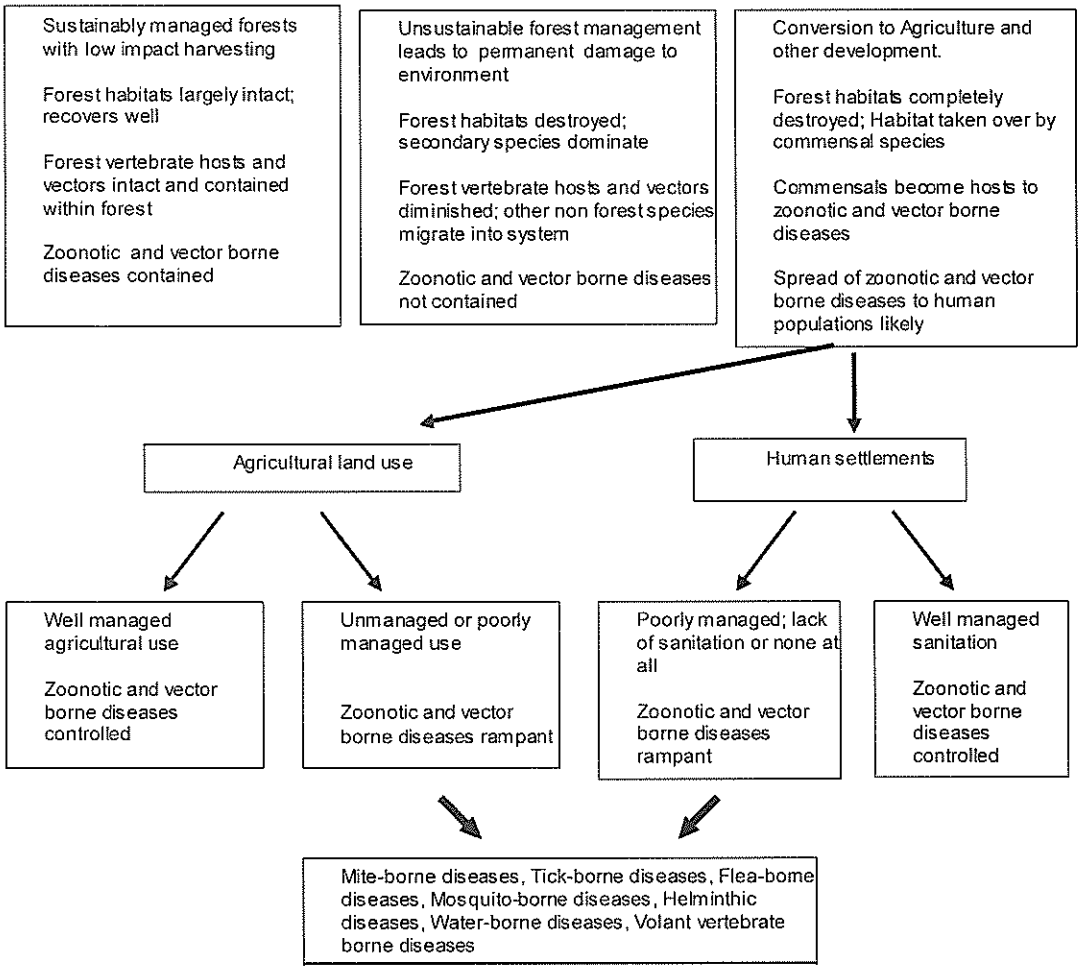

Figure 1. Spread of zoonotic and vector borne diseases in different land use regimes.

RECOMMENDATIONS FOR RESEARCH

In the world of research funding in this country, 'Basic Research' enjoys the same status that leprosy had in society before sulphone was introduced. Like leprosy, basic research is something that should not be discussed, much less being considered for possible support. Sometimes, however, if such research is coated with the right high glamour ingredients such as DNA and subtly slipped into the arena of 'Applied Research', funds can be obtained. In biological research without understanding the basic elements of the subject, in this case the ecology of the animal concerned, it will be difficult to understand its functions, interactions and relationship with its natural environment. In this context, apart from some of the large mammals such as the elephant, tiger, rhinoceros, tapir, guar where in-depth ecological studies have been carried out, ecological information of other species like the panther, clouded leopard, bear, serow, etc is still lacking. At present our knowledge of these mammals is limited to occasional reports of their occurrence in inventory work. The lack of field biological information of the latter group is because they are considered to be unimportant and of low priority. By the time government agencies and conservation groups from local and non-governmental organizations begin to realize the importance of these 'second class animals', it may be too late to launch practical measures to conserve viable breeding populations of these species.

Additional information that will help conserve these species would be knowledge of their beneficial roles in nature and forest ecosystems. This lack of information is primarily due to lack of basic research. It is sad that when such research is proposed it is more often not funded because such research does not fall within the category of 'Applied Research'. This classification of science into basic and applied may be a dangerous distinction as it could sideline work which is considered of no immediate 'commercial' applied value, until a problem of medical or economic importance emerges. By that point of time, however, the ecological parameters may have been destroyed and an ecological solution to the problem may no longer be possible. For example, the transmission of dengue to humans may have started with forest destruction but this was not envisaged until the disease was endemic in human populations.

Habitat preservation

The first step must be to identify all the remnant habitats that are deemed critical, work out their areas and distributions and then gazetting them as legally protected areas. This is a logical step as failure to do so may result in their destruction/development even before we are able to document their importance towards the preservation of the Peninsula's mammalian fauna. The lowland dipterocarp forest may already be so reduced that it may no longer serve this purpose.

Basic research on the lowland and hill forest species and ecosystems

The next stage would be to mount a programme on basic research into the different families of mammals so that their status, the roles they play in the forest ecosystem and the needs (if any) to ensure their long term survival. To undertake such basic research requires the collaboration of a wide range of expertise and scientists.

International and regional collaboration

We must be realistic that much of the expertise required might not be available within Malaysian institutions. There are scientists with wide experience who are experts in small carnivores (felids, viverids, mustelids), rodents (nocturnal sciurids – flying squirrels), insectivores (ground shrews, moles). We must be prepared to accept such foreign expertise to work alongside us under a partnership paradigm that is both fair and honorable. Their roles are to not only assist us financially, but also to train our young scientists in biological field research.

A good example is the Malaysian Bat Research Group (Texas Tech University/Zoology Department/ UKM/DWNP) that has been active since the early 1990s. During this period here, one significant result was the training and financing of more than 10

graduate students to MSc and PhD level. Malaysian science was enhanced in the areas of bat taxonomy and systematics, ecological research and related studies. Among the spin offs was an educational workshop on bats for children and the promotion of the value of bats as important components of the ecosystem among the Orang Asli community.

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Effects of prebiotic chocolates on some physicochemical properties, intestinal microflora of rat's digesta and their serum profile

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Abastract This study was carried out to evaluate the effectiveness of newly developed prebiotic milk chocolate (MC) and dark chocolate (DC) in stimulating the growth of Bifidobacteria and Lactobacilli in large intestine of male Sprague Dawley rats (n = 72) compared to control (C) diet. Body weight gains, weight of liver, kidney and small intestine did not differ significantly ($p > 0.05$) in all the test groups fed with prebiotic chocolates. Moisture content and weight of rats' digesta were greater ($p < 0.05$) in groups fed 5% prebiotic milk chocolate (PM) and 5% prebiotic dark chocolate (PD) with positive correlation ($p < 0.01$). There was significantly higher count ($p < 0.01$) of bifidobacteria and lactobacilli at week-4 (end of experiment) with a gradient decreased in *E. coli* count for PM group compared to C group. A lower serum total cholesterol level was also observed in PM, PD and IN group compared to C group. Triacylglycerol, glucose, uric acid and total protein contents of the serum were not significantly different ($p > 0.05$) in all test groups. In conclusion, inulin in milk and dark chocolate were able to exert prebiotic effect with contribution in lowering serum cholesterols and did not influence body weight of rats.

Keywords Bifidobacteria – chocolate – gastrointestinal tract – Inulin – rats' serum

INTRODUCTION

The importance of prebiotics as nondigestible dietary components that pass through the digestive tract to the colon, were identified as fermentable materials by the microbiota thus capable to stimulate the proliferation and/ or activity of the endogenous desirable bacteria such as bifidobacteria and lactobacilli [1]. Other researchers have demonstrated higher amount of *Bifidobacterium spp.* in the cecum of rats caused by ingestion of prebiotics may prevent colonization of potential pathogen by lowering pH of the gastrointestinal tract [2]. Meanwhile, effects of dietary fibre rich oat based products resulted in no differences in the body weight and weight of liver, spleen and stomach of experimental rats but significantly greater in wet contents of their caecum and colon together together with significantly lower pH values after six weeks of experiment [3].

Inulin (prebiotic) is one of the well known dietary fibers that decreases fat absorption, provides roughage preventing constipation and lowering the blood cholesterol [4]. Studies in experimental animals suggested that dietary inulin, like other soluble dietary fibers, may modulate the concentration of serum lipids [5, 6]. Therefore, we have selected inulin in this study to be added into milk and dark chocolates (as a partial sugar replacer and as added dietary fibre) in order to increase the nutritional value of the chocolates which is considered as top antioxidant food (Source: Data from the US Department of Agriculture and the Journal of the American Chemical Society). The amount of inulin used in this

study was actually selected due to scientific evidence [7] on its dose effect relationship (5g/day). Our preliminary study in vitro (unreported data), also showed that 5g/L inulin in basal medium supported significant growth of selected bifidobacteria strains. The incorporation of inulin in milk and dark chocolate has not yet been previously reported.

Our aim in this study was to determine and to compare among prebiotic milk and dark chocolates (with inulin) and control chocolates (without inulin) in affecting the proliferation of bifidobacteria as well as other microbial populations in experimental animals (rats). The nutritional value of the chocolate to the rats was also observed through their body weight gain and serum cholesterol level.

MATERIALS AND METHODS

Source of chocolates and prebiotic

Newly developed prebiotic milk chocolate (MC) and dark chocolate (DC) by Malaysian Cocoa Board (International Application No: PCT/ MY 2007/000023) were prepared using cocoa solids, cocoa butter, milk powder, emulsifier, a flavor component and sweeteners (inulin and isomalt). The control milk and dark chocolates were prepared containing sucrose as a sweetener. Cocoa liquor purchased from Selbourn Food Services at Pelabuhan Klang, Malaysia, full cream and skimmed milk from Promac Enterprises Sdn. Bhd., cocoa butter from Malaysia Cocoa Manufacturing Sdn. Bhd., isomalt from Nutrisweet & Food Specialties Sdn. Bhd., and inulin extracted from chicory root (Sensus, The Netherlands).

Rats and experimental design

Male Sprague Dawley rats (n = 72, 3 to 4 weeks of age) were obtained from Laboratory Animal Centre, Medical Faculty of Universiti Malaya, Kuala Lumpur. All treatments and diets were reviewed and approved by the Animal House of University Kebangsaan Malaysia, Bangi, Selangor. After arrival, rats were quarantined for a week (wk) during which they were fed with rodent chow (control) diet (Gold Coin, Malaysia). The compositions of a control diet are crude protein (23%), crude fibre (5%), crude fat (3%), moisture (13%), ash (8%), calcium (1.2%), phosphorus (1.0%), nitrogen free extract (49%) and additives (vitamins A, D_3, E, C, K, B_{12}, thiamine, riboflavin, panthothenic acid, niacin, pyridoxine, choline, antioxidant and microminerals).

Rats were then divided randomly into 6 experimental groups (n=12) and were housed individually under controlled temperature (25°C) with a 12 hr light:dark cycle and fed about 15g control diet followed by experimental diets for 4 weeks (oral feed). Rats had free access to water and were weighed weekly throughout the study. Treatments given to experimental rats were as follows and labeled as: C = control diet; IN = control diet + 5% inulin; SD = control diet + 5% control DC; SM = control diet + 5% control MC; PD = control diet + 5% prebiotic dark chocolate; and PM = control diet + 5% prebiotic milk chocolate (5% w/w doses in rats were equivalent to approximately 5g/ day in human diet).

Sampling and analytical procedures

The body weight of each rat was recorded weekly. On the final day of the experimental period, wk-1(7d),-2(14d),-3(21d) and 4-(28d), rats were fasted overnight and killed by chloroform inhalation. The entire large intestine (cecum and colon) was tied off immediately after dissection to avoid leakage of cecal contents (separate out from small intestine) due to relaxed gut muscle upon death [8]. Internal organs of each rat such as liver, kidney and small intestine were also removed and weighed.

The content (digesta) was removed from the untied large intestine and weighed before microbial analyses. The pH of the samples was measured with a portable pH meter (Thermo Electron, Beverly, MA) against a standard buffer of 4 and 7 pH. The moisture in digesta was determined after drying in a fan ventilate oven (Memmert, Germany) at 80°C until a constant weight was achieved [8].

Tied large intestine samples were cut and collected weekly from each rat in separate sterile pack before extracted for microbial analyses within 1 hr of collection. One g of digesta from the large intes-

tine was homogenized with 9 ml of sterile peptone water (Merck). Ten- fold serial dilutions of each sample were poured plated in triplicate. Plate count agar (Oxoid) was used for counting total aerobes and total anaerobes. Tryptone Peptone Yeast agar (TPY agar) (Sharlau) was used for bifidobacteria, MRS agar (Oxoid) for total lactobacilli and Chromocult agar (Merck) for *E.coli*. Plates of total anaerobes, bifidobacteria and lactobacilli were incubated anaerobically at 37°C for 48 hr in anaerobic jars with gas generating kit. Plates for the enumeration of total aerobes and *E.coli* were incubated at 37°C for 48 hr. Bifidobacteria, lactobacilli as gram positive and *E.coli* as gram negative rods in each type of plate were identified using Gram Staining, Biolog (MicroLog 3, USA) and API systems (BBL Crystal, Becton, Dickinson, USA) before proceeded with TPY, MRS and Chromocult as a selective media.

Blood samples of fasting rats were collected immediately in sterile tubes by heart puncture (before rats were killed), and left to stand for 30 min at room temperature (25°C) to coagulate before being centrifuged for 20 min at 3,000 x g. The serum samples were measured for total cholesterol, triacylglycerols, glucose, uric acid and total protein by immunoturbidimetric assay (Hitachi 902 Automatic Analyzer) using commercial kits and quality control materials (Roche Diagnostics) as described by Katja [9].

Statistical analyses

All data obtained were analysed using SPSS Inc. software (version 14.0). One-way ANOVA was used to determine a significant difference between means of the dietary groups and sampling sites with a significance level of $p < 0.05$. Tukey-test was used to perform multiple comparisons between each experiment with $n = 12$.

RESULTS

Rats body and organs weight

During the 4-wk experiment, body weight (BW) gains did not differ significantly ($p > 0.05$) among the rats fed with control and the different experimental diets as shown in Table 1. However, rats fed with control diets showed highest BW gain (75.83 ± 10.11g) compared to IN, SD, SM, PD and PM group. All rats were generally healthy throughout the feeding trial period. No significant difference ($p>0.05$) was also observed in their weight of liver, kidney and small intestine of control and treated rats.

Weight, pH and moisture content of digesta

All treated groups had no significant difference ($p > 0.05$) in the weight of digesta at wk-2 and wk-4, but wk-3 the weight was significantly different ($p < 0.05$) in all rats with the highest digesta's weight (5.31 ± 0.22g) was recorded in rats fed with PM,

Table 1. Body weight (BW) gain, weight of liver, kidney and small intestine before and after treatment of rats fed with control and experimental diets (n = 12).

		Weight (g)		
Treatments*	BW gain (g/rat/wk)	Liver	Kidney	Small intestine
Control	75.83[a]	7.39[a]	1.63[a]	6.32[a]
IN	43.33[a]	6.78[a]	1.54[a]	5.93[a]
SD	46.67[a]	7.09[a]	1.61[a]	6.67[a]
SM	63.33[a]	7.71[a]	1.56[a]	5.97[a]
PD	53.33[a]	6.71[a]	1.50[a]	6.36[a]
PM	60.00[a]	7.80[a]	1.70[a]	6.26[a]

[a] Means in the same row and column followed by same alphabet is not significantly different at $p > 0.05$.

* Treatments: C = Control rats given 15g basal diet, IN = Rats given 15g basal diet + 5% inulin, SD = Rats given 15g basal diet + Control DC (5%sucrose), SM = Rats given 15g basal diet + Control MC (5%sucrose), PD = Rats given 15g basal diet + 5%prebiotic dark chocolate, PM = Rats given 15g basal diet + 5%prebiotic milk chocolate.

at wk-4, followed by rats fed with PD (4.62 ± 0.78g) and IN (4.24 ± 0.72g) diets. Despite no significant different ($p > 0.05$) in pH of the digesta at wk-2 and wk-4, at wk-3 showed significant different of pH ($p < 0.05$) in all treated groups. C groups had the highest pH value (7.14 ± 0.46) at wk-4 compared to other groups.

All experimental rats showed significant difference ($p < 0.05$) in moisture content (Table 2) especially at wk-4 although no significant different ($p > 0.05$) observed in all treated and C groups from wk-2 until wk-3. At wk-4, rats fed with PM had the highest moisture content (6.94 ± 0.50 %) in digesta followed by the PD (5.33 ± 0.93%) and IN (4.11 ± 1.05%) compared to the control diet (3.28 ± 1.07%).

Microbial populations

Tukey test showed that number of total aerobe (Fig. 1) was significantly lower ($p< 0.05$) at wk-1 while higher total anaerobe count at wk-1 and wk-2 (Fig. 2). Total aerobes ranged from 6.19 to 8.27 $\log_{10}$ cfu/g wet digesta (wk-1 to wk-4). Rats fed with SD diet showed lowest concentration of total aerobe count at wk-4 compared with control groups. An increased trend of total anerobe was observed from wk-1 (6.82 ± 0.15 $\log_{10}$ cfu) until wk-3 (8.10 ± 0.00 $\log_{10}$ cfu) for C groups. Figure 2 shows total

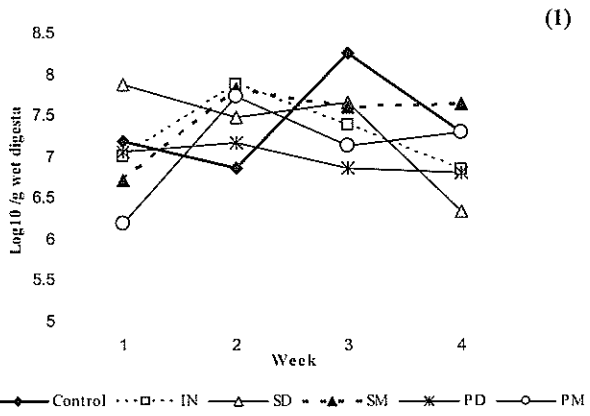

Figure 1. Population pattern of Total Aerobes in digesta of rats fed with control and experimental diets from wk- 1 until wk- 4*. * Treatments: C = Control rats given 15g basal diet, IN = Rats given 15g basal diet + 5% inulin, SD = Rats given 15g basal diet + Control DC (5%sucrose), SM = Rats given 15g basal diet + Control MC (5%sucrose), PD = Rats given 15g basal diet + 5%prebiotic dark chocolate, PM = Rats given 15g basal diet + 5%prebiotic milk chocolate.

anaerobe ranged from 6.82 to 8.43 $\log_{10}$ cfu/g wet digesta for all groups. Rats consuming SM diet had lower total anaerobe count at wk-4 (7.24 ± 0.43 $\log_{10}$ cfu) compared with other groups.

There was significant different in bifidobacteria and lactobacilli count ($p < 0.05$) at wk-2 and wk-4. A gradient decreased in bifidobacteria count (Fig. 3) was observed from wk-1 to wk-4 for SD, SM and control diet groups. Numbers of bifidobacteria

Table 2. Moisture (%), pH and weight of digesta of rats fed with control and experimental diets from wk- 2 until wk- 4 (n = 3).

		Treatments*					
	Wk	Control	IN	SD	SM	PD	PM
Moisture (%)	Wk2	2.42^{a}	2.47^{a}	3.81^{a}	3.40^{a}	2.05^{a}	3.65^{a}
	Wk3	1.68^{a}	4.52^{a}	1.87^{a}	3.15^{a}	3.78^{a}	2.03^{a}
	Wk4	$3.28^{a,b}$	$4.11^{a,b}$	$3.34^{a,b}$	1.67^{a}	$5.33^{a,b}$	6.94^{b}
pH	Wk2	5.83^{a}	7.25^{a}	6.48^{a}	6.04^{a}	6.43^{a}	6.52^{a}
	Wk3	$6.98^{b,c}$	$6.77^{a,b,c}$	7.15^{c}	$6.20^{a,b}$	5.98^{a}	$6.61^{a,b,c}$
	Wk4	7.14^{a}	7.04^{a}	6.00^{a}	5.99^{a}	6.08^{a}	6.26^{a}
Digesta	Wk2	3.13^{a}	3.27^{a}	3.88^{a}	3.10^{a}	2.55^{a}	4.00^{a}
	Wk3	$2.75^{a,b}$	4.60^{b}	2.54^{a}	$3.49^{a,b}$	$4.03^{a,b}$	2.34^{a}
	Wk4	3.30^{a}	4.24^{a}	3.56^{a}	3.24^{a}	4.62^{a}	5.31^{a}

[a-c] Means in the same row followed by different alphabet is significantly different at $p< 0.05$

* Treatments: C = Control rats given 15g basal diet, IN = Rats given 15g basal diet + 5% inulin, SD = Rats given 15g basal diet + Control DC (5%sucrose), SM = Rats given 15g basal diet + Control MC (5%sucrose), PD = Rats given 15g basal diet + 5%prebiotic dark chocolate, PM = Rats given 15g basal diet + 5%prebiotic milk chocolate.

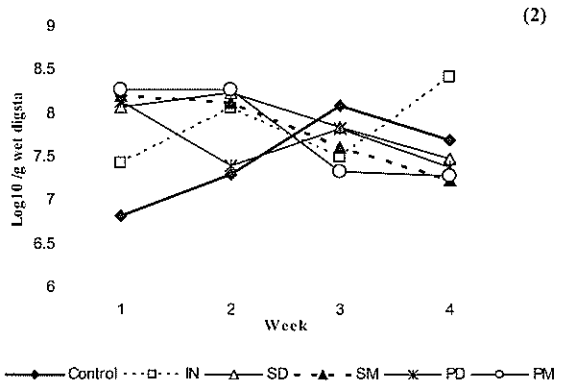

Figure 2. Population patternofTotalAnaerobesindigesta of rats fed with control and experimental diets from wk-1 until wk-4*.

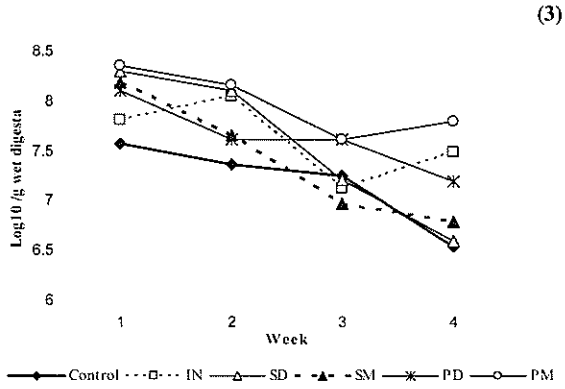

Figure 3. Population pattern of Bifidobacteria in digesta of rats fed with control and experimental diets from wk-1 until wk-4*.

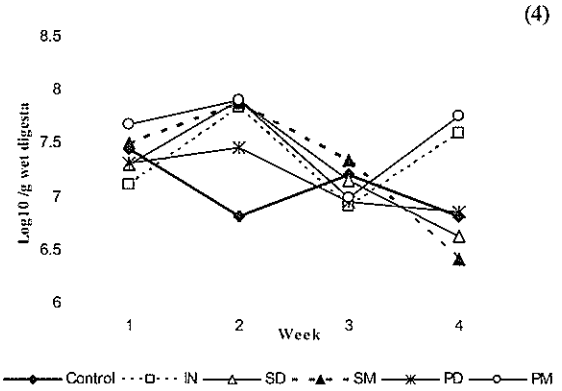

Figure 4. Population pattern of Lactobacilli in digesta of rats fed with control and experimental diets from wk-1 until wk-4*.

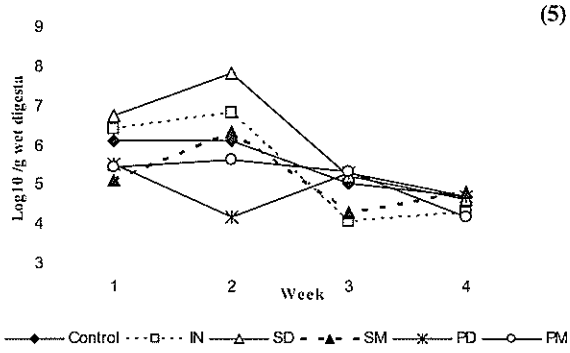

Figure 5. Population pattern of *Escherichia coli* in digesta of rats fed with control and experimental diets from wk-1 until wk-4*.

ranged from 6.54 to 8.36 $\log_{10}$ cfu/g wet digesta. There was an increased in bifidobacteria count from wk-3 to wk-4 for IN and PM group by 0.4 and 0.2 $\log_{10}$ cfu/g wet digesta respectively. Rats fed with PM diet had the highest bifidobacteria count at wk-2 (8.17 ± 0.07 $\log_{10}$ cfu) and wk-4 (7.80 ± 0.24 $\log_{10}$ cfu) in comparison to rats fed with control diet. Similar result was also recorded in lactobacilli count at wk-2 and wk-4 for PM group compared with C group. The lactobacilli count (Fig. 4) ranged from 6.63 to 7.91 $\log_{10}$ cfu/g wet digesta. An increased in lactobacilli count from wk-3 to wk-4 was observed for PM and IN group by 0.8 and 0.7 $\log_{10}$ cfu/g wet digesta respectively compared with those fed with control diet.

Despite the significant different in bifidobacteria and lactobacilli count ($p < 0.05$) at wk-2 and wk-4, the number of *E.coli* showed significantly lower count ($p < 0.05$) at wk-2 and wk-3. A gradient decreased in *E.coli* count (Fig. 5) was recorded in PM group. Treated and control rats showed an average count of *E.coli* between 4.07 to 7.84 $\log_{10}$ cfu/ g wet digesta from wk-1 to wk-4.

Serum profile

The serum profile of treated and control rats, which comprised of cholesterol, triacylglycerols, glucose, uric acid and total protein contents at the beginning and the end of each treatment period is shown in Table 3. No significant different ($p > 0.05$) in serum cholesterols, triacylglycerols, glucose, uric acid and total protein contents of rats fed with control diet and other experimental diets. However, Paired Sample *t*-test showed significant difference ($p < 0.05$)

for cholesterol level before (wk-0) and after (wk-4) treatment compared with other serum parameters. Rats fed with IN (1.32 ± 0.12 mmol/L), PD (1.42 ± 0.07 mmol/L) and PM (1.38 ± 0.00 mmol/L) showed much lower concentrations in cholesterol when compared with the C group (1.61 ± 0.14 mmol/L) after the final day of treatment. IN, PM and C rats showed a decreased in serum triacylglycerol level when compared between wk-0 and wk-4. Despite an increased level of glucose in rats fed with IN, SD, PD and PM; rats fed with control diet (5.57 ± 0.59 mmol/L) and SM (6.30 ± 0.06 mmol/L) diet showed a decreased in glucose concentration. There was a significant positive correlation ($p < 0.05$) between glucose concentration before and after treatment. Nevertheless, uric acid and total protein concentration showed an increased trend for most of the groups.

DISCUSSION

Pearson correlation test showed there was a positively significant correlation at $p < 0.01$ between moisture content and digesta weight of the rats, in which the moisture content of digesta increases together with the increased in digesta weight for all rats. Rats fed with PM and PD diet had higher digesta moisture content compared to the control. Liong [8] also reported a significant increase in moisture content of rats fecal when fed with diets containing prebiotics. It was stated that osmolality (concentration of particles in solution) is a major factor in determining water content of lumen where osmolality would be increased substantially by the presence of the unfermented carbohydrate used. Our finding is also similar to Zenon [10] where they found an increase in mass of cecal content of rats supplemented with 5% and 10% inulin. It was reported that the prebiotic have a bulking effect due to the increase in microbial biomass as a result of fermentation [1, 11].

The prebiotic chocolate given to the experimental rats in this study showed an insignificantly decrease in pH value (below pH 7) compared to rats fed with control diet. This was also in agreement with Zenon [10]. This indicated that the chocolate containing inulin does not inhibit inulin fermentation in the rats' cecum. The effect was desirable

Table 3. Serum cholesterol, triglycerides, glucose, uric acid and total protein of rats fed with control and experimental diets before (wk-0) and after given experimental diets (wk-4) (n = 3).

		Treatments*					
	Wk	Control	IN	SD	SM	PD	PM
Cholesterol (mmol/L)	0	1.67[a]	1.60[a]	1.62[a]	1.39[a]	1.65@	2.06[a]
	4	1.61[a,A]	1.32[a,A]	1.52[a,A]	1.48[a,A]	1.42[a,A]	1.38[a,A]
Triacylglycerol (mmol/L)	0	0.94[a]	1.13[a]	0.89[a]	0.48[a]	0.59[a]	0.96[a]
	4	0.62[a]	0.73[a]	1.05[a]	0.94[a]	0.91[a]	0.92[a]
Glucose (mmol/L)	0	5.73[a]	7.37[a]	8.17[a]	8.10[a]	8.07[a]	5.33[a]
	4	5.57[a]	7.53[a]	8.73[a]	6.30[a]	8.57[a]	7.10[a]
Uric acid (μmol/L)	0	68.60[a]	58.60[a]	42.20[a]	65.47[a]	84.63[a]	119.40[a]
	4	137.73[a]	91.70[a]	85.30[a]	62.50[a]	100.63[a]	90.93[a]
Total protein (g/L)	0	62.03[a]	56.70[a]	57.77[a]	57.63[a]	54.27[a]	60.70[a]
	4	63.57[a]	58.70[b]	61.10[a]	59.77[a]	58.00[a]	59.73[a]

[a] Means in the same row followed by same lowercase alphabet is not significantly different at $p > 0.05$.
[A] Means in the same column followed by different uppercase alphabet is significantly different at $p < 0.05$.
* Treatments: C = Control rats given 15g basal diet, IN = Rats given 15g basal diet + 5% inulin, SD = Rats given 15g basal diet + Control DC (5%sucrose), SM = Rats given 15g basal diet + Control MC (5%sucrose), PD = Rats given 15g basal diet + 5%prebiotic dark chocolate, PM = Rats given 15g basal diet + 5%prebiotic milk chocolate.

because a decrease in colonic pH might reduce the risk of developing colon cancer [12]. In this study, there was negatively significant correlation ($p<0.05$) observed between pH of digesta and moisture content where as the pH of digesta decreased, the moisture content of the digesta increased. Other reports suggested that the pH reduction is a consequence of the prebiotic metabolism by the fermentative bacteria [13, 14]. This effect is beneficial for the organism as it constitutes an ideal medium for the development of the bifidogenic flora and at the same time limits the development of pathogenic bacteria [14].

This might be true in the case of our study, due to significant prebiotic effect of selective stimulation of the growth of lactobacilli and bifidobacteria species observed in rats fed with milk chocolate supplemented with inulin (PM) compared with rats fed with inulin only (IN) and the C group. Scientific studies by Japan scientists indicated that consumption of prebiotic shifts the balance of microflora in the intestine towards greater population of bifidobacteria and other beneficial microorganisms even in the absence of probiotic added to the diet [14]. There was significant increased ($p < 0.05$) of bifidobacteria count from wk-3 to wk-4 for IN and PM group by 0.4 and 0.2 $\log_{10}$ cfu/g wet digesta respectively. Similar significant ($p < 0.05$) increasing trend was also observed in the lactobacilli count. However, Roberfroid [15] stated that log increase in bifidobacteria counts do not necessarily correlate with daily doses administered but rather depends on the initial numbers of bifidobacteria. An increase of bifidobacteria less than one log unit is difficult to assess, and the absolute increase in number of bifidobacteria is likely to be less important than the statistical significance of the increase [15].

Elmer [16] stated that the presence of bifidobacteria and lactobacilli may act as wards regulating the activity of the disease causing organisms such as *E. coli*, Clostridia, Salmonella, etc. either by helping to resist infection, or by creating conditions which reduce the number of pathogenic bacteria. Lactobacilli have been reported to inhibit the binding of enteropathogenic *E.coli* to intestinal cells [17].

This study showed significant reduction ($p < 0.05$) for the serum total cholesterol (32.7%) in rats fed with PM after final day of experiment (28d) when compared to the C group (3.5%). A report published by Sangeeta [18] on fermented food based on cereals containing *S. boulardi* and *L. casei* showed significant reduction of only 19% total cholesterol (after 42d). Other report also showed that some lactobacilli when grown under proper conditions (anaerobic and presence of bile) will remove cholesterol from lab media [19] but a controversy still exist as to whether the bacteria can exert cholesterol lowering action in vivo. However, no significant different ($p > 0.05$) of triacylglycerols recorded in all groups except slight decreased in PM group (0.96 to 0.92 mmol/L). In contrast to our findings, daily feeding of oligofructose to rats at 10% dose level resulted in significantly lowering serum triacylglycerols after just one week of feeding as well as reduced serum phospholipids and total cholesterol level [5]. Kok [20] postulated that the lower glucose and insulin level that were found after feeding 10g/100g oligofructose to rats contributed to the reduced hepatic fatty acid and triacylglycerols synthesis. However, our finding showed that there was positively significant correlation ($p < 0.05$) between glucose at wk-0 and wk-4 with increasing trend after 28d although insignificant different of glucose level ($p > 0.05$) was observed in all groups.

Our study confirms that inulin in milk and dark chocolate was able to exert prebiotic effect by elevating the bifidobacteria and lactobacilli counts in line with inulin supplementation alone above the sucrose containing chocolates. The prebiotic chocolates also did not influenced body weight gain of the rats but did contribute on lowering their serum cholesterols better than control diet. Indirectly, this will help in increasing the health benefits of chocolate and related products.

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Role of chalcones in prevention and treatment of cancer

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Abstract Cancer is a hyperproliferative disorder that involves dysregulation of as many as 300 different genes. Numerous agents have been identified which mediate tumorigenesis including tobacco, cancer-causing viruses, and environment pollutants. Similarly agents have also been identified which suppress this process of tumorigenesis are named as chemopreventive agents. The link between the agents that cause cancer and those that prevent cancer appears to be the nuclear transcription factor NF-κB, which is activated by carcinogens and suppressed by chemopreventive agents. This review describes molecules belonging to the chalcone family which are derived from stembark of cashews (*Semecarpus anacardium*), the heartwood of *Dalbergia odorifera*, and the traditional Chinese and Tibetan medicinal herbs *Caragana jubata* and *Rhus verniciflua*, seeds of *Amomum subulatum*, fruits of *Alpinia rafflesiana*, *Alpinia henryi*, *Alpinia conchigera* Griff , *Boesenbergia pandurata* and other zingiberous plant species. These agents can suppress NF-κB activation and thus have potential for inhibiting carcinogenesis and tumorigenesis.

Keywords chalcones – butein – NF-κB – chemoprevention

INTRODUCTION

Natural dietary agents including those from fruits, vegetables, and spices have drawn a great deal of attention from both the scientific community and the general public owing to their demonstrated ability to suppress cancers. The questions that remain to be answered are which component of these dietary agents is responsible for the anti-cancer effects and what is the mechanism by which they suppress cancer? Dietary agents consist of a wide variety of biologically active compounds that are ubiquitous in plants, many of which have been used in traditional medicines for thousands of years. As early as 2500 years ago, Hippocrates recognized and professed the importance of various foods both natural and those derived from human skill in the primary constitution of the person.

Most of the currently available targeted therapies for age-associated chronic illnesses are not very effective, exhibit numerous side effects, and for more than 80% of the world's population, are too expensive. Thus alternatives, which are desirably should be less expensive, more efficacious and exhibit minimum toxicity, are needed. Numerous epidemiological, clinical and experimental evidence suggest that fruits and vegetables can lower the incidence of most diseases, including cancer [1]. Neither the active principles in fruits and vegetables nor its their mechanisms of action is are fully understood.

ROLE OF NF-κB IN CANCER

NF-κB represents a group of five proteins namely, c-Rel, RelA (p65), RelB, NF-κB1 (p50 and p105), and NF-κB2 (p52) [2]. The NF-κB proteins are regulated by inhibitors of the IκB family of anchorin domain–containing proteins, which includes IκBα, IκBβ, IκBγ, IκBε, Bcl-3, p105, and p100 [2]. In an inactive state, NF-κB is sequestered in the cyto-

plasm as a heterotrimer consisting of p50, p65, and IκB subunits. Most carcinogens, inflammatory agents, and tumor promoters, including cigarette smoke, phorbol ester, okadaic acid, H_2O_2, and tumor necrosis factor (TNF), have been shown to activate NF-κB (Fig. 1). In response to an activation signal, the IκB subunit is phosphorylated at serine residues 32 and 36, ubiquitinated at lysine residues 21 and 22, and degraded through the proteosomal pathway, thus exposing the nuclear localization signals on the p50-p65 heterodimer. The p65 is then phosphorylated, leading to nuclear translocation and binding to a specific sequence in DNA, which in turn results in gene transcription. The phosphorylation of IκBα is catalyzed by IκBα kinase (IKK), which is essential for NF-κB activation by most agents. IKK consists of three subunits, IKK-α, IKK-β, and IKK-γ (also called NEMO) [3]. Gene deletion studies have indicated that IKK-α is essential for NF-κB activation by most agents [3]. The kinase that induces the phosphorylation of p65 is controversial, but IKK-α, protein kinase C, and protein kinase A have been implicated [3; 4; 5]. NF-κB has been shown to regulate the expression of several genes whose products are involved in tumorigenesis. These include antiapoptotic genes (e.g., cIAP, survivin, TRAF, Bcl-2, and Bcl-xL); COX-2, matrix metalloproteinase-9 (MMP-9); genes encoding adhesion molecules, chemokines, and inflammatory cytokines; and cell cycle regulatory genes (e.g., cyclin D1 and c-myc) [6] (Fig. 2).

TARGETING NF-kB BY CHALCONES

Chalcones are a group of aromatic enones that forms the central core to a variety of important biological compounds. They are also intermediates in the biosynthesis of flavonoids, which are substances widespread in plants and with an array of biological activities. The simplest chalcone can be prepared by an aldol condensation between a benzaldehyde and an acetophenone in the presence of sodium hydroxide as a catalyst.

Chalcones are frequently obtainable from plant sources, including the stembark of cashews *Anarcardium occidentale* L, the heartwood of *Dalbergia odorifera*, and the herbs *Caragana jubata* and *Rhus verniciflua*.

Chalcones have been reported to possess a variety of biological properties, including analgesic, antioxidant, antifungal [7], antibacterial, antiprotozoal [8; 9], gastric "protectant", antimutagenic, and antitumorogenic [10] and anti-inflammatory properties [11; 12; 13], although the mechanisms of action of this class of compounds are not yet fully understood.

Below is a description of role of chalcones in prevention and treatment of cancer.

Butein

Butein (3,4,2',4'-tetrahydroxychalcone) has been identified from numerous plants including stembark of cashews the heartwood of *Dalbergia odorifera*,

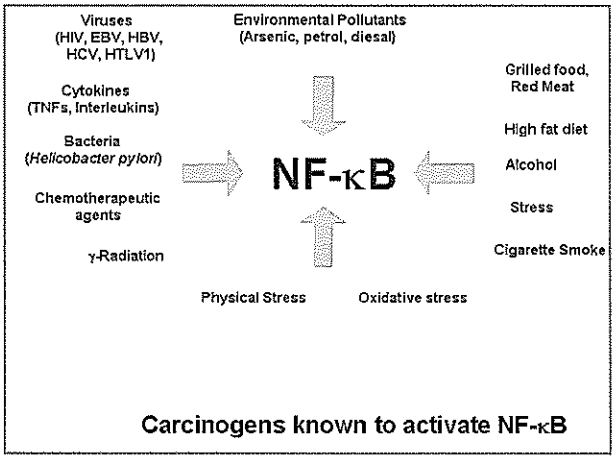

Figure1. Various activators of NF-κB.

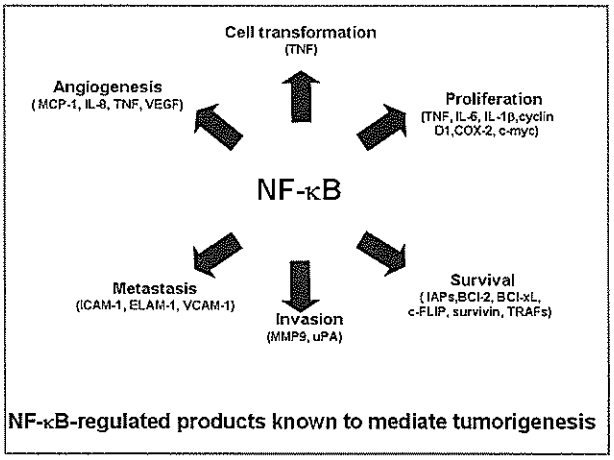

Figure2. Various gene products regulated by NF-κB.

Caragana jubata and *Rhus verniciflua*. Evidence suggests that in rodents, butein suppressed phorbol ester-induced skin tumor formation [14]; abrogated carrageenan-induced rat paw edema [15], exhibited hypotensive effects [16], inhibited diabetic complications [17], reduced antibody-associated glomerulonephritis [18], suppressed liver fibrosis induced by carbon tetrachloride [19], and ameliorated renal concentrating ability in cisplatin-induced acute renal failure [20]. *In vitro*, butein suppressed the proliferation of a wide variety of human tumor cells, including breast carcinoma [21; 22], colon carcinoma [23; 24], osteosarcoma [25], lymphoma [26; 27], acute myelogenous leukemia [28], melanoma [29] and hepatic stellate cells [30].

It is not fully understood how butein mediates the anti-inflammatory and cytotoxic effects. However, investigators have reported that butein modulated the expression of epidermal growth factor receptor tyrosine kinase [31; 32], adhesion molecules [18], Bcl-2 [28; 29], iNOS [23], phosphodiesterase IV [33; 34], glutathione S-transferase (GST) [35], hemeoxygenase-1 [30], the uptake of Ca^{2+} [36], 12 lipooxygenase (12-LOX), ornithine decarboxylase [14], angiotensin-converting enzyme [16], and tissue inhibitor of metalloproteinase (TIMP)-1 [19; 37]. Additionally, butein has been shown to suppress the activity of aldolase reductase [17], glutathione reductase [38], tyrosinase [39], COX-1 [15], farnesyl protein transferase [23], and aromatase [21]; mediate antioxidant effects [40; 41] inhibit drug export [42]; suppress the GST-mediated conjugation of chlorambucil with GSH [43; 44] and chelate iron and copper [45].

Because butein exhibits anti-inflammatory and antiproliferative effects and suppresses the expression of adhesion molecules, iNOS, Bcl-2, TIMP-1, and 12-LOX, all of which are known to be regulated by the transcription factor NF-κB, therefore, butein must mediate its effects by modulating the NF-kB activation pathway, which has been closely linked to inflammation, tumorigenesis, proliferation, invasion, angiogenesis, and metastasis, and is activated in response to various inflammatory agents, carcinogens, tumor promoters, and growth factors [6; 46; 47]. We reported that butein suppressed NF-κB activation pathways activated by a variety of agents through the direct inhibition of IκBα kinase (IKK) which led to the suppression of NF-κB-regulated gene products and the enhancement of apoptosis induced by inflammatory cytokines [48]. Overall, studies of Pandey et al, 2007 suggest that butein's anticarcinogenic, anti-inflammatory, and apoptotic effects are mediated through the inhibition of the IKK-induced NF-κB pathway, that is activated by a wide variety of carcinogens and inflammatory agents. Hence, butein has full potential to be used in prevention or treatment of cancer.

Cardamonin

Cardamonin (22 ,42 -dihydroxy-62 -methoxychalcone) is a naturally occurring chalcone. This compound has been isolated from the seeds of *Amomum subulatum*, fruits of *Alpinia rafflesiana*, *Alpinia henryi*, *Alpinia conchigera* Griff, *Boesenbergia pandurata* and from other zingiberous plant species. Some of these species growing in northeastern Vietnam have been used in Vietnamese traditional medicine for the treatment of inflammatory diseases. Cardamonin demonstrated significant anti-mutagenic effects upon activation of heterocyclic amines [49], caused vasorelaxation of rat mesenteric artery via NO-mediated processes [50] and has anti-inflammatory properties [51]. Cardamomin was identified as an inhibitor of NF-κB activation. This compound inhibited the induced activation of NF-κB and expression of NF-κB target genes such as COX-2 and iNOS in LPS-stimulated RAW264.7 cells [52]. Furthermore, pretreatment of cardamomin protected the mice from death caused by LPS administration, suggesting its potential for anti-inflammatory and anti-carcinogenic agent.

Flavokavains

Flavokavains are the active constituents of kava which is extracted from the rhizome of the pepper plant, *Piper methysticum*, and that species is found in Polynesia, Melanesia, and Micronesia. Kava extracts contain, the chalcones flavokavain A,

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Sweetpotato as a staple food crop: opportunities and challenges

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Abstract Malaysia faces the current shortage of rice in the world market. Being only 70% self-sufficient in this staple, it may be prudent to look at other sources of carbohydrate. One promising source is sweetpotato, which can be more nutritious than white rice in having more vitamins (especially A, C and E) and certain minerals (Ca, Mg, K and Cu), as well as high dietary fibre. Many of these vitamins and nutrients, as well as its low glycaemic index, bring a lot of health-benefits to those who eat sweetpotato. The main shortcoming of this crop is in having less of several essential amino acids (ile, leu, met and val), although its protein content can be as high as in white rice.

Sweetpotato is highly versatile in having a wide cultivation zone, ranging from temperate to humid tropics, and in being used in various food preparations. In Malaysia, being able to adapt (with the required agronomic amendments) to marginal soils such as *bris*, tin-tailings, acid sulphate soils and drained peat, it can compete favourably with other crops for arable land. A range of available processing technologies also provide the means for increasing its popularity as a supplementary staple, so that consumers do not tire of eating sweetpotato just boiled, steamed, baked or fried. Thus, there are ample opportunities for investing in sweetpotato for down-stream processing into wholesome food products.

Keywords sweetpotato – *Ipomoea batatas* – staple food – root crop – food security – Malaysia

INTRODUCTION

Rice is a staple food in Malaysia – as in many other Asian countries. It plays an important role in the human diet as a source of carbohydrate which provides energy to fuel physical activities as well as metabolic and anabolic processes in the body. Currently, Malaysia is about 70% self-sufficient in rice production, having to import the remaining 30%. This has worked very well until recently with the unprecedented (since World War II) worldwide shortage in rice supplies, leading to prices escalating by two to three times. This crisis in rice supply is the culmination of several factors (mainly, [1]):

- The world is consuming more rice than is produced. This trend began as early as 2000 but was not reflected by any price increase (until recently) because of the masking effect of released stockpiles. For example, in Malaysia, our 3-month stockpile was reduced to two weeks.
- The growing demand for rice is a result of developing economies and population growth. Other than China and India with their substantial rice-eating populations, Africa now imports almost one-third of the total volume of rice traded.
- There has been almost no yield increases in major rice-growing countries in Asia over the last five to six years, coupled with little expansion in rice production areas.
- Planthopper outbreaks have been reported (since 2005) in Vietnam, China, Indonesia, Korea and Japan due to higher temperatures (resulting from climate change). This has led to a ban on exporting rice in Vietnam, one of the major rice-exporters.
- Natural disasters, such as droughts in India and China, typhoons in the Philippines, flooding in

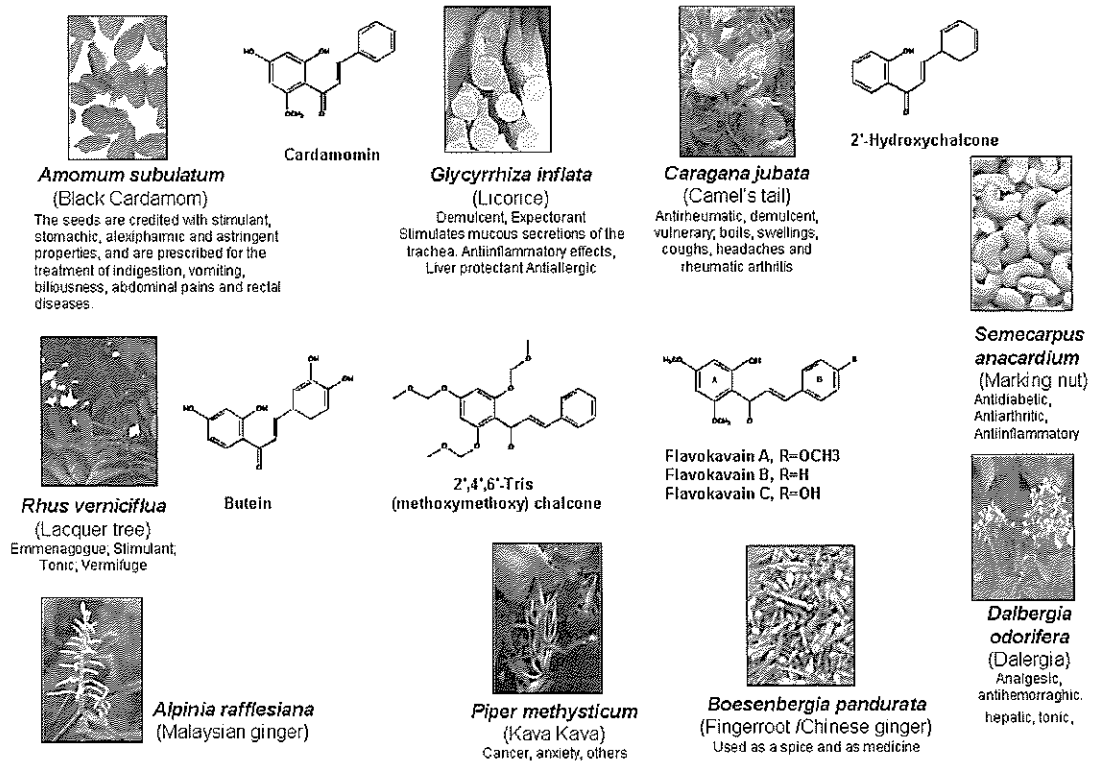

Figure 3. Sources of chalcones and their inhibition on NF-κB.

flavokavain B, and flavokavain C, as well as traces of the piperidine alkaloids pipermethysticine and awaine [53; 54]. Kavain and methysticin are the major components of the roots and rhizomes, whereas their dihydro derivatives constitute the major components of the leaves. Kava has been reported as a potent ethno-pharmacological remedy for over 3000 years [55; 56; 57; 58]. These chalcones have also been historically used as treatment for gonorrhoea, rheumatism, bronchitis, asthma, as well as stomach aches and headaches. Kava was adopted as a medicinal plant by Europeans soon after its discovery in the Pacific Islands. It was used in Germany for treatments of urinary ailments and gonorrhoea as early as 1850 and the first pharmacological preparations were found in Germany in the 1920s offered as tincture for use as a mild sedative and hypersensitive herb. The alkaloids, which were recognized as the compounds responsible for rare but severe liver damage observed with chronic consumption of some commercial kava herbal supplements [53] are mainly present in the leaves and stem peelings of kava [56]. The anti-inflammatory effects of kava-chalcones (flavokavains) leading to inhibition of NF-κB activation induced by TNFα in human leukaemia cells, suggest its anti-inflammatory activity is mediated through NF-κB pathway [59].

2'-Hydroxychalcone

Recently, synthetic 2'-hydroxychalcone has been shown to be a potent antioxidant, anti-inflammatory agent. It inhibits lipid peroxidation and is antitumorogenic [60]. Having the hydroxyl group at the *ortho* position on the benzene ring of chalcone increases its antioxidant property compared with other substituted chalcones. Very little is known in regard to its mechanism of action. Studies [61] showed that 2'-hydroxychalcone blocks the adhesion of neutrophils to endothelial monolayers by preventing TNF-α- and LPS-induced up-regulation of cell adhesion molecule expression on endothelial cells. Moreover, this chalcone inhibits TNF-α-in-

duced cell adhesion molecule expression by blocking the activation of NF-κB in endothelial cells.

It is also reported that 22 -hydroxychalcone derivatives inhibit TPA-induced prostaglandin E2 (PGE2) production through the inhibition of COX-2 induction in rat peritoneal macrophages [62], inhibits LPS-induced production of NO and TNF-α, murine macrophage cell line RAW 264.7 suggesting its potential as anti-inflammatory agent.

2',4',6'-tris(methoxymethoxy)chalcone (TMMC)

Synthetic 22, 42, 62 -tris(methoxymethoxy) chalcone inhibits LPS-induced NO production via sustained HO-1 induction. That is also mediated by NF-kB, suggesting its potential for anti-inflammatory and anticancer [30].

3,4-Dihydroxychalcones

A novel series of 3,4-dihydroxychalcones was synthesized to evaluate their effects against 5-lipoxygenase and cyclooxygenase. The 2',5'-disubstituted 3,4-dihydroxychalcones with hydroxy or alkoxy groups exhibited optimal inhibition of cyclooxygenase. 2',5'-dimethoxy-3,4-dihydroxychalcone inhibited cyclooxygenase to the same degree as flufenamic acid and 5-lipoxygenase, more than quercetin. Finally, these active inhibitors of 5-lipoxygenase inhibited arachidonic acid-induced mouse ear edema more than phenidone [63].

Licochalcone A

Licochalcone A, 3-a,a-dimethylallyl-4,4'-dihydroxy -6-methoxychalcone, from the root of *Glycyrrhiza inflata* Beta (Leguminosae) (Xin-jiang liquorice) showed anti-inflammatory action towards mouse ear edema induced by arachidonic acid (AA) and 12-*O*-tetradecanoylphorbol 13-acetate (TPA) by topical application. Anti-tumour promoting action of licochalcone A was also observed *in vivo* for mouse skin papilloma initiated by dimethylbenz[a]anthracene (DMBA) and promoted by TPA. It inhibited *in vitro* 32Pi-incorporation to phospholipids in HeLa cells promoted by TPA [64].

CONCLUSION

Natural dietary agents including from fruits, vegetables, and spices have drawn a great deal of attention from both the scientific community and the general public owing to their demonstrated ability to suppress cancers. The questions that remain to be answered are which of these dietary agents are responsible for the anti-cancer effects and what is the mechanism by which they suppress cancer.

Because NF-κB is critical transcription factors that regulate the production of various proinflammatory proteins and cytokines in activated macrophages during the process of inflammation, the inhibition of this transcription factors might be an effective therapeutic approach for inflammatory diseases such as rheumatoid arthritis. It is possible that chalcone derivatives are lead compounds for novel anti-inflammatory drugs having inhibitory activity on the production of various inflammatory mediators such as PGE2, NO and cytokines.

Chalcones continue to attract considerable scientific attention because of their association with a variety of biological activities. The question may be asked as to why chalcones are associated with this plethora of biological activities. Its origin as natural products may be a contributory factor. More importantly, the structural features of chalcones – presence of a reactive enone moiety - and its relative flexibility compared to other related natural products like flavonoids, may predispose the template to interactions with diverse receptors and enzymes. The ease with which chalcones are synthesised further enrich the structural diversity of the template through the introduction of features normally associated with ligand-receptor interaction, namely hydrophobic groups, hydrogen bond donor and acceptor features. It has been noted that the chalcones normally exert their activities in the middle to low micromolar range, with fewer examples of activity in the nanomolar range. Skilful structural manipulation of the chalcone framework may yet narrow its range of biological activity and enhance its potency for a targeted pharmacological profile.

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Heavy metals in shrimp pond sludge, water and muscles

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Abstract Sarawak is a key producer of cultured black tiger shrimp (*Penaeus monodon*). However, knowledge on heavy metals in shrimp pond is scarce in literature. Therefore, concentrations of selected heavy metals (Zn, Cu, Mn, Cd) in shrimp pond sludge, pond water, shrimp feed, and shrimp muscles from two farms in different locations were investigated. Results indicated that heavy metals concentrations of sludge, though elevated, did not exceed the standard established. Cu in the sludge was significantly correlated with the feed and the soil. Pond water heavy metals were less than 0.2 mg/L except for Mn in Farm 2. Zn in the water was highly correlated with Zn in the feed. Even though Cu in the Farm 2 feed was six times that of Farm 1, there was no significant difference in the muscles. Furthermore, though Mn in sludge and water of Farm 2 were 4 and 20 times that of Farm 1, muscle Mn from Farm 2 was less than Farm 1 and concentrations were low. In the shrimp muscles, Zn concentration was the highest followed by Cu. The muscle concentrations of all heavy metals except Mn were not significantly correlated with that in the feed, sludge or water indicating the ability and selectivity of shrimp to regulate heavy metals. The concentrations of Zn, Cu and Cd in the shrimp muscle did not exceed the maximum permissible limits of the Malaysian Food Act.

Keywords shrimp culture – heavy metals – aquaculture – pond sludge

INTRODUCTION

One of the fastest growing components of the aquaculture industry in the world is shrimp farming [1]. The major shrimp producing countries are in Asia, where the black tiger (*Penaeus monodon*) is the predominant farmed species and Central America where the western white (*Penaeus vannamei*) is the predominant species [2]. Malaysia produced a total of 12,000 metric tons of tiger shrimps in 2000. From 1998 to 2004, there was a drastic increase in shrimp culture in Sarawak due to the abundant aquatic resources, underdeveloped coastal land and relatively unpolluted water resources, cheap and easily trained labour forces, existing government policies and natural conditions [3, 4]. In 2004, there were 317 licensed farm operators operating 2,105 ponds covering an area of 1,125 hectares.

Shrimp pond nutrients and organic matter have been extensively studied [4-6]. However, not as much studies were conducted on heavy metals content of pond sludge, feed and shrimp muscles. Other than the feed input, other factors may contribute to the presence of heavy metals in the pond. They include the water used for the shrimp culture which is usually from the estuary where the ponds are located and the shrimp farm management practices. Coastal zones and estuaries are also regions frequently contaminated with heavy metals. A wide variety of chemicals and biological products were used in semi-intensive and intensive South-East Asian shrimp of heavy metals were present in the shrimp muscles and pond water [10]. Some of the heavy metals such as zinc (Zn), copper (Cu) and manganese (Mn) are essential nutrients for the shrimp but others such as Cd have no known use in physiological process [8]. The objective of this study

was to investigate the presence of selected heavy metals in pond water, pond sludge, shrimp feed and shrimp muscles.

MATERIALS AND METHODS

Sample collection and preservation

Water and sludge samples were collected from shrimp culture ponds during harvesting between September and December 2005 from two locations, Telaga Air (Farm 1) and Asajaya (Farm 2). Sampling of sludge in the ponds was made at four equal distances from the centre to the edge of the pond by using polyvinyl chloride (PVC) tubes. Pond size ranged from 0.18 to 0.90 ha. Shrimps and the feeds were obtained from the farm operators and soil samples were also collected from the farm areas. All samples were placed in plastic bags and transported in an icebox to the laboratory. Water and sludge samples were acidified with concentrated nitric acid to pH less than 2, but not lower than 1.75 [11] and stored in acid rinsed polyethylene bottles at 4 °C.

Samples preparation and analysis

Shrimp muscle tissue was processed according to [10]. Shrimp muscle tissue was dried at 80 ° C for about 24 hours in an oven. Two grams of the dried samples were digested in 10 mL of concentrated nitric acid at 60 ° C for 30 min. Subsequently, the sample solution was cooled to room temperature and 2 mL of 30% hydrogen peroxide was added before analysis of heavy metals.

The sludge sample was digested according to [12]. One gram of sludge was weighed and 10 mL of 1:1 (concentrated HNO_3:H_2O) was added before heating to 95 ° C for 15 min. The sample was cooled and another 5 mL of concentrated HNO_3 was added and refluxed for 30 min. As the solution metals content were analysed. The shrimp feed and soil samples were pulverized using mortar before digestion as described for sludge. Water sample was digested according to Standard Methods [13] before filtration and analysis for heavy metals. All digested samples were analyzed using Flame Atomic Absorption Spectrophotometer (FAAS, Perkin Elmer, 3110).

Statistical analysis

Statistical analyses were performed using SPSS version 14.0. Univariate analysis of variance was performed to find possible differences between farms, layers and distances. Independent *t*-test was used to compare concentrations of heavy metals in muscle, feed, and soil between the farms. Bivariate correlation was conducted to investigate relationship between heavy metals in muscles, sludge, and water with feed and soil.

RESULTS

The mean concentrations of heavy metals in the sludge at different distance from the centre of the ponds are shown in Table 1. For Farm 1, the concentration of heavy metals were in decreasing order of Zn>Mn>Cu>Cd. All heavy metals analyzed in the sludge were the lowest in concentration at the

Table 1. Mean concentrations and standard deviations of selected heavy metals in sludge at different distance from the centre of the pond in Farm 1 and 2.

Farm	Distance (m)	Mean concentration (mg/kg dw)			
		Zn	Mn	Cu	Cd
1	0.0	73.7±6.9^{a}	71.0±10.8^{a}	21.6±2.6^{a}	3.0±0.7^{a}
	16.8	80.6±11.2^{a}	68.4±8.0^{a}	17.1±5.2^{bc}	3.7±0.9^{a}
	33.6	57.3±9.8^{b}	56.8±12.7^{b}	19.5±6.5^{ab}	3.1±1.1^{a}
	50.4	50.8±9.3^{b}	44.3±8.1^{c}	13.6±2.8^{c}	1.8±0.7^{b}
2	0.0	91.8±7.8^{a}	233.3±20.2^{a}	35.8±2.1^{a}	2.7±1.4^{a}
	7.5	72.4±5.1^{b}	212.2±9.8^{b}	36.0±1.7^{a}	1.1±1.1^{a}
	15.1	70.9±1.8^{b}	212.9±22.3^{b}	33.6±3.1^{a}	2.4±1.3^{a}
	22.6	84.9±4.0^{c}	164.4±18.6^{c}	35.6±3.3^{a}	2.2±1.2^{a}

*For each farm, values in the same column with the same superscript are not significantly different at 5% level.

point furthest from the centre of the ponds and the highest concentration occurred within 16.8 m from the centre. For Zn and Mn, the concentration decreased from the centre to the edge. However, for Cu and Cd, the trend was not as distinct.

For Farm 2, the concentration of Mn was the highest, exceptionally high actually, followed by Zn, Cu and Cd. The highest concentration of heavy metals was in the inner half of the radial distance from the centre of the pond. However, statistical analysis indicated that for Cu and Cd, the concentrations were independent of distance from the centre of the pond. Comparing between the two farms, the mean concentrations of all heavy metals were significantly different. Farm 2 sludge shows higher Zn, Mn and Cu whereas Farm 1 has higher mean Cd.

Comparing between mean Cd in the sludge with that of the feed, all the heavy metals are lower in the sludge except for Mn of Farm 2 and Cu of Farm 1 (Table 1 and 2). In both farms the mean concentrations of the heavy metals in the sludge were significantly higher than that of the soil except for Cd which were not significantly different ($P = 0.9$) (Table 2 and 3). Feed had the highest concentration of Zn followed by Mn and Cu (Table 2). Cd was detected in small concentrations in all types of samples with the feed showing significantly higher concentration than those in muscle, soil and sludge ($P < 0.001$). In both farms, the heavy metals in the feed were higher than that in the soil.

In shrimp muscles, all the heavy metals detected were below that in the feed and sludge and Zn concentration was the highest followed by Cu, Mn, and Cd (Table 4). Even though Mn was high in the sludge and feed, it was very low in shrimp tissue and did not reflect the high concentrations in the feed and sludge. Concentrations of Zn and Mn in the muscles were significantly different from sludge, soil and feed. However, Cu concentration in muscle was not significantly different from the feed and soil and Cd in the muscle was significantly lower than the feed ($P < 0.0005$) but not significantly different from soil ($P = 0.57$) and sludge ($P = 0.90$).

As for the distribution of heavy metals in the shrimp pond water in the two farms, except for Mn in Farm 2, all heavy metals were below 0.2 mg/L (Table 5). The highest concentration of Mn in sludge of Farm 2 was also observed in the water. In both farms, the concentration of Cu was the lowest instead of Cd as occurred in the sludge. Comparing between the two farms, Mn and Cd in pond water were significantly different ($P < 0.0005$, $P = 0.037$).

Even though Cu concentrations of Farm 2 feed were six times higher than that of Farm 1 the concentrations in the shrimp muscle were not significantly different ($P = 0.742$) (Table 2 and 4). Furthermore, muscle Zn concentrations between the two farms were not significantly different even though in the feed, they were significantly different. Correlation analysis indicated that in the muscle, only Mn was significantly correlated with the feed ($r = 0.915$, $P = 0.011$). Zn in the water was highly correlated with Zn in the feed ($r = 0.936$, $P = 0.006$). Cu in the sludge was strongly correlated with the feed ($r = 0.986$, $P < 0.0005$) and significantly correlated with the soil ($r = 0.834$, $P = 0.039$).

Table 2. Heavy metals in shrimp feeds and soils from the farming areas.

Heavy Metal	Feed (mg/kg dw)		Soil (mg/kg dw)	
	Farm 1	Farm 2	Farm 1	Farm 2
Zn	95.1±0.6^a	64.9±3.7^b	30.6±1.6^a	27.3±2.5^a
Mn	65.5±2.2^a	48.1±1.6^b	22.3±0.6^a	20.8±7.4^a
Cu	9.8±0.6^a	57.5±2.2^b	4.8±0.6^a	7.1±0.9^b
Cd	6.3±1.1^a	5.3±1.1^a	2.4±0.6^a	1.6±0.7^a

*For each heavy metal in feed or soil, values in the same row with the same superscript are not significantly different at 5% level.

Bangladesh and the recent Cyclone Nargis in Myanmar, have led to great yield losses.

- Increases in petroleum prices have driven up fertilizer (e.g. urea) prices and freight costs, which have an effect on the price of traded rice.
- There have been cutbacks in rice production by some exporting countries to free land for biofuel production. For example, Thailand was planning to reduce by half its rice exports before the rice crisis struck.

The Malaysian government is currently taking urgent action to increase local rice production by expanding the cultivated area as well as establishing new granary areas. This, especially the latter which requires infrastructural development, will take time. In the meanwhile, we should explore the possibility of supplementing rice intake by considering other carbohydrate sources such as sweetpotato.

NUTRITIONAL VALUE OF SWEETPOTATO

For too long root crops have remained underground; it is now time to reveal their qualities. As their economic parts develop below the soil surface, there is no clear maturation period for root crops unlike cereals such as rice. Nevertheless, the optimum time to harvest root crops has been established for different species.

Another significant difference from cereals is that at harvest, the moisture content of root crops is quite high (about 60-65%) compared to around 15-17% in cereals. Thus, cereals do not require a great deal of effort in drying after harvest.

To make more meaningful comparisons, data on the nutrient compositions of sweetpotato and cassava – the two leading root crops in Malaysia – have been converted to a dry basis (at 12% moisture content) for their comparison with white rice (Table 1). It is evident that:

- Energy from the root crops is just slightly lower than from rice;
- Protein content of cassava is low, but that in sweetpotato is comparable to the content in white rice (unpolished brown rice will have a higher protein content);
- White rice has practically no dietary fibre (the high-fibre bran having been removed during polishing), whereas dietary fibre content in sweetpotato is quite high;
- White rice has low contents of Ca, Mg, K and Cu, but has a higher Mn content than the root crops;
- Vitamin content in white rice is also low – especially for vitamins A, C (completely absent) and E, thiamin, riboflavin, niacin and total folate;
- The low protein content of cassava is also reflected in its contents of amino acids. Sweetpotato is better but is much lower in certain amino acids (e.g. the essential amino acids ile, leu, met and val) compared with white rice; and
- Orange-fleshed sweetpotato contains a high level of ß-carotene. When ingested, ß-carotene is converted to vitamin A in the body.

Thus, root crops need not be inferior in nutritive value compared to rice, particularly in terms of providing energy, dietary fibre, minerals and vitamins. Dietary fibre has positive effects against diabetes, constipation, and possibly colorectal cancer. Potassium is effective against hypertension, and provides protection against cardio-vascular disease. Calcium builds strong bones, while iron is important for women in their child-bearing years. Vitamins A, C and E are powerful antioxidants which act against defects in the unborn foetus, certain cancers and the ravages of ageing. (Another powerful antioxidant is anthocyanin, the presence of which is manifested as purple in sweetpotato). Vitamin E also reduces the risk of cardio-vascular disease and stroke.

The biggest shortcoming of the root crops is their deficiency in certain essential amino acids. However, Malaysians have access to many other protein sources than rice itself, so it is not a big concern.

When comparing the root crops with white rice in terms of their glycaemic indices or GI (Table 2), it may be seen that cassava and sweetpotato have low GI (46 and 50, respectively) whereas white rice has a GI of 70. GI measures the rate at which an in-

DISCUSSION

The elevated concentration of heavy metals in the pond sludge when compared with the soil of the area was also reported in Australia where pond bottom soil had higher content of heavy metals than pond wall soil [5]. The elevated sludge Cu content is most likely due to Cu being added to eliminate external protozoan, control bacterial diseases, inhibit phytoplankton growth and induce moulting in shrimps [14] and also from the feed as Cu in the sludge was positively correlated with the feed. The concentrations of Cu and Zn in the sludge were lower than that reported in Australia (45 mg/kg vs 85 mg/kg) [5]. Zn concentration of 50.8-91.8 mg/kg in the pond sludge falls in the range of 40.8-3448 mg/kg reported in India [9]. The sludge Cd content could be attributed to the feed, soil and the pond water as pond water originated from the estuary which received runoff from the populated watershed. The presence of Cd in feed was also reported in poultry, pig and cattle feed [15]. Soil could account for some of the Cd as Cd concentration in the soils of the world was reported to range from <0.005 to 8.1 mg/kg [16].

When compared with the maximum possible concentrations of heavy metal in sludge for agricultural use or disposal on land established by the Department of Environment (DOE) [17], mean Zn, Cu and Cd from both farms did not exceed the standard (Table 3). According to the USEPA guideline classification [18], the pond sludge Zn content of both farms falls under unpolluted state but Cu of Farm 2 is classified as slightly polluted (Table 3).

The exceptionally high Mn concentration in the water and sludge of Farm 2 is most likely due to the added Mn in the form of potassium permanganate to control external protozoan, metazoan parasites and bacterial and fungal diseases [6, 19]. Cd was detected in the farm soils possibly due to both natural and anthropogenic sources. Cu concentration in the pond water was lower than Cd due to selectiv-

Table 3. Heavy metals in sludge compared with guidelines of heavy metals in sludge or sediment.

Heavy Metal	DOE Limit[1]	USEPA Guideline Classification[2]			Present Study (mg/kg)	
		Unpolluted	Slightly polluted	Heavily polluted	Farm 1	Farm 2
Zn	200	<90	90-200	>200	65.6±6.9[a]	80.0±8.7[b]
Cu	80	<25	25-50	>56	18.0±2.6[a]	35.2±1.0[b]
Cd	3	N.A	N.A	>6	2.9±1.1[a]	2.1±0.6[b]

N.A: Not available. *For each heavy metal, values in the same row with the same superscript are not significantly different at 5% level. [1]Maximum possible concentrations of heavy metal in sludge for agricultural use or disposal on land [18][2]. USEPA guideline classification value for sediment or metal concentration in sludge [19].

Table 4. Comparison of results in shrimp muscles in this study with maximum permissable level of heavy metals in shrimo according to Malaysian Food Act 1983 and literature values.

Heavy Metal	Malaysian Food Act [26]	Literature values		Present study (μg/g)	
		[8]	[10]	Farm 1	Farm 2
Zn	40	16.07	1.8-3.0	13.1±0.3[a]	12.9±0.3[a]
Mn	NA	0.49	0.2-0.3	0.5±0.1[a]	0.3±0.1[b]
Cu	30	7.23	0.3-0.7	1.4±0.1[a]	1.3±0.2[a]
Cd	1	ND	0.1-0.3	0.4±0.1[a]	0.4±0.1[a]

ND=below detection limit (Cd<0.05). For each heavy metal, values in the same row with the same superscript are not significantly different at 5% level.

Table 5. Heavy metals in pond water compared with effluent discharge standard to Malaysian inland waters.

Heavy Metal	Maximum Permitted value Standard B†	Present study (mg/L)	
		Farm 1	Farm 2
Zn	1.0	0.13±0.01[a]	0.10±0.01[a]
Mn	1.0	0.12±0.02[a]	2.62±0.03[b]
Cu	1.0	0.04±0.01[a]	0.03±0.01[a]
Cd	0.02	0.09±0.01[a]	0.03±0.01[b]

*For each heavy metal, values in the same row with the same superscript are not significantly different at 5% level. †Environmental Quality (Sewerage and Industrial Effluents) Regulations 1979 (Standard B), Department of Environment, Malaysia.

ity of clay minerals and hydrous oxide adsorbents in soil and sediment where Cu is more strongly adsorbed than Zn, and Cd being more weakly adsorbed are more labile and bioavailable [20]. Cd in the pond water could originate from the soil, feed, or the water which originated from the estuary which received domestic and sewage effluents and runoff from agricultural areas. Compared with the effluent discharge standard specified by the Environmental Quality Act 1974, the Environmental Quality (Sewerage and Industrial Effluents) Regulations 1979 (Standard B), for discharge downstream of any raw water intake, Zn and Cu of both farms and Mn of Farm 1 did not exceed the standard established. However, Mn of Farm 2 and Cd of both farms exceeded the discharge standard established (Table 5).

Zn, Mn and Cu were observed in shrimp muscles as they are recommended dietary supplements for shrimp as essential nutrients [21]. The high Zn concentrations in shrimp muscles could be due to the importance of Zn as an activator of numerous enzymes present in the hepatopancreas and in the midgut gland of crustaceans [22]. The muscle Zn and Mn concentrations corresponded to the report of [8] (Table 4). However, the Cu concentration in the muscle in the present study was much less thanthat in their study. Comparisons with results of two farms in Sabah, Mn concentrations were similar but Zn and Cu in the muscle in the present study were higher than those reported [10]. Cd was detected in the shrimp muscles in the present study. This observation was also reported in Sabah where muscle Cd concentrations were reported to be 0.1-0.3 μg/g [10] and in India (0.11-3.2 μg/g) [9]. Furthermore, in the pond water in those studies, Cd concentrations were reported to be 0.4-0.5 and 0.04-0.10 mg/L respectively in Sabah and India. Cd was reported in hepatopancreas (1.39 μg/g) but not in the muscles of farmed *Penaeus monodon* in Australia [8]. The occurrence of Cd in muscle of shrimp could be due to the feed and habitat evidenced by the presence of Cd in the feed, sludge and pond water and the mechanisms of aquatic organism acquiring heavy metals from food, suspended particles or directly from water [22]. According to studies, both colloidally-bound metals and ionic forms were reported to be taken up by juvenile brown shrimp (*Penaeus aztecus*) and that the primary pathway for the movement of metals from solution to the tissue was across the gill epithelium where negatively charged binding sites of phosphate, carboxyl, amino, and sulphate groups attracted the metal cations [23]. However, even though feed Zn and Cu concentrations of the two farms were significantly different, their concentrations in the shrimp muscle were not significantly different. Furthermore, heavy metals in the muscles were not correlated with that of water and this was also reported in Sabah [10]. Heavy metals in muscles were also reported to be not significantly correlated with those in sediment and water in India [9]. These could be due to the ability of shrimps to regulate the concentrations of heavy metals as shown by experiments on accumulation and discharge of selected heavy metals [24]. It was found that Cu granules accumulated were excreted in faeces. Other than faeces, crustaceans were known to eliminate excess heavy metals through

urine and moulting and through gills [25]. Compared with Malaysian Food Act [26], the level of Zn, Cu and Cd detected in shrimp muscles from the two farms studied did not exceed the maximum permissible level.

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Water-blown flexible polyurethane foams: Effect of water content on mechanical, morphology and thermal characteristics

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Abstract The effect of water content on mechanical, morphology and thermal characteristics of flexible foam was investigated. The water-blown flexible polyurethane foams were made based on ratio 1:1 (w/w) mixture of palm oil-based polyester polyol with number average molecular weight (Mn) of 5646 and commercial polyether polyol with molecular weight of 5000, isocyanate index 1.00 and varying amounts of 2, 3 and 4 pph of water contents. Water content of 2 pph produced the highest density foam (57.89 kg/m^3) with the lowest hysteresis (23.64 %), the highest tensile strength (0.097 MPa) and elongation at break (138%). However, higher amount of water contents produced foams with higher open call contents (~98%) and more thermally stable.

Keywords Density – elongation at break – flexible polyurethane – hyteresis – isocyanate index – palm oil-based polyester polyol – tensile stress – thermal characters – water contents

INTRODUCTION

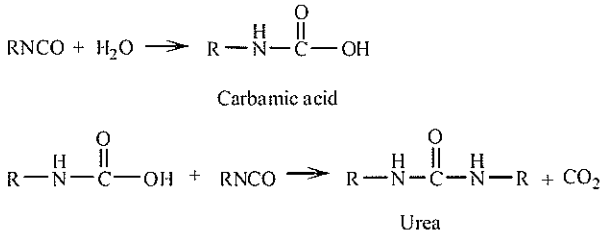
Production of flexible polyurethane foam involves the use of a number of chemicals as additives, such as tin catalyst, amine catalyst, silicone surfactants and blowing agent. Conventionally, high volatile liquid such as chlorofluorocarbons (CFCs) was used as blowing agent in polyurethane foam production. CFCs, which were used to lower the foams' density and to soften the flexible foams were the primary contributor to the depletion of ozone in the stratosphere [1]. Such depletion causes increased harmful ultraviolet radiation to the earth and led to adverse health and biological effects, for instance, including the increase in skin cancer. In addition, CFCs being strong 'greenhouse' gases could lead to global warming.

The issue of CFCs emission into the atmosphere has received increasing worldwide concern. Various approaches are being investigated to reduce the use of CFCs by replacement with other high volatile blowing agents such as methylene chloride [2], hydrochlorofluorocarbon (HCFC) [3], chlorodifluoromethane (R22) [4] and others. However, methylene chloride is suspected for being carcinogenic, and almost every other auxiliary blowing agent poses safety concerns because of flammability properties or toxicity. Therefore, there is a need to find alternative blowing agent for the production of flexible slab-stock polyurethanes.

One of the alternatives is to use carbon dioxide generated from the reaction of water with isocyanate as a sole blowing agent [5]. Motte [6] also reported the study of flexible polyurethane molded foam by using water as blowing agent in comparison to molded foam prepared by CFC-11. Foam blown with carbon dioxide showed improved properties in low density foam [6].

Water is introduced into polyurethane to expand the cellular structure of the foam by contributing to the blowing reaction. On the other hand, water will also lead to gelling reaction in polyurethane [7]. This is attributed to the formation of unstable carbamic acid yielded from the reaction of water and reactive isocyanate. The unstable carbamic acid will then splits off into carbon dioxide and amine. The amine will instantly react with isocyanate to form symmetrical urea (Scheme 1). Physical cross-linking in the foam will arise from the phase separated urea hard segments [8] and will produce foams with different physical properties.

In this work, the effect of water contents in the formulation on the physical properties, morphology and thermal characters of flexible polyurethane foams was studied. The water contents were altered by 2 pph, 3 pph and 4 pph of water.



Scheme 1. The emission of carbon dioxide and formation of urea by the reaction of isocyanate with water.

MATERIALS AND METHODS

Materials

Palm oil-based polyester polyol B1 with number average molecular weight (Mn) of 5646 (Hydroxyl value = 89.91 mg KOH/g sample) and commercial polyether polyol with molecular weight of 5000 (Hydroxyl value = 38.12 mg KOH/g sample) were obtained from AOTD, MPOB. Additives: NIAX stannous octoate catalyst (G.E. Silicone), NIAX A33 triethylenediamine (TEDA) catalyst (G.E. Silicone), NIAX dimethylethanolamine (DMEA) cross-linker (G.E. Silicone), Niax silicone surfactant L580 (G.E. Silicone) and toluene diisocyanate (TDI) (Miliken Chemical), of industrial grade were used.

Foam preparations and evaluations

Equivalent ratio 1:1 (w/w) of palm oil-based polyester polyol and commercial polyether polyol, stannous octoate catalyst, TEDA catalyst, DMEA cross-linker, silicone surfactant and water as blowing agent were mixed together in a plastic cup and stirred vigorously at 8000 rpm by a mechanical stirrer until the mixture became creamy. After that, the stirring was stopped before adding TDI and the stirring process continued at the same speed for another 10-15s. The liquid mixture was then poured into a plastic box (18 x 12 x 8 cm) and left to rise and cured at room temperature (25°C) for 7 days before all the mechanical measurements were carried out.

Density measurement

The test specimens (100 x 100 x 50 mm) were weighed to determine the density in kilogram per cubic meter. Three specimens were tested and the average value was reported.

Compression stress and hysteresis test

The test was conducted according to DIN 53577 Compression of Flexible Cellular Materials Test. Foams with dimension 50 x 50 x 50 mm were compressed between two flat plates at the rate of 100 mm/min. The instrument used was Zwick Universal Testing Machine (United Kingdom) with crosshead monitor and compression platens as the grip. Based on the stress against strain curve graph, compressive stress and hysteresis were recorded.

Tensile stress and elongation at break

The test was conducted according to ASTM D3574 Foam Tension Test E. Foams were cut to flat sheets with 12.5 $\pm$ 1.5 mm thickness and cut into dumbbell shape according to Test Methods D412. The test was conducted by using Zwick Universal Testing Machine (United Kingdom). The specimens were gripped by two screw-type flat plate grips and pulled at a speed of 500 $\pm$ 50 mm/min. The tensile stress and the elongation at break were recorded.

Tear resistance test

The test was conducted according to ASTM D3574 Foam Tear Resistance Test F. The specimens with

specific shape were clamped to the jaws of the test machine, Zwick Universal Testing Machine (United Kingdom). The specimen block was pulled by the jaw diagonally at the speed of 500 ± 50 mm/min. Maximum force was recorded and the tear strength was calculated as follow: Tear Strength, N/mm = F/T; where, F = force, N and T = thickness, mm.

Percentage of open cell contents

Micromeritics Accu Pyc 1330 Pycnometer (United States) was used to determine the volume of the sample block based on the pressure change of nitrogen on a calibrated volume. Two sample cubes with the size 25 x 25 x 25 mm were placed into the Pycnometer cylinder and the gas displacement volume (V_{p1}) was determined. Then, each cube was cut into eight smaller cubes and placed back into the cylinder to determine the second gas displacement volume (V_{p2}). Volume of open cells was determined using the formula $V_{oc} = 31.25 - 2V_{p1} + V_{p2}$ and percentage of open cell contents, $\% V_{oc} = (V_{0c}/31.25) \times 100$, in which 31.25 was the geometric volume of the samples.

Microphotograph of the foam cell

A magnified cross section microphotograph on the flexible foam cell was done using the Olympus A70 Provis Microscope (Japan) that was connected to a programmable computer. The shapes of the cells were compared.

Thermal gravimetric analysis (TGA)

About 4-5 mg of foam was used in the analysis of the thermal gravimetric (TG) profile with the TA Instruments SDT 2960 Simultaneous DSC-TGA Analyzer (Newcastle, DE). Pyrolysis was started from 25°C and ended at 800°C with the heating rate of 10°C/min under a flow of nitrogen gas at 100 mL /min. The results were plotted as percentage of weight loss against temperature in degree Celsius.

RESULTS AND DISCUSSION

Alteration in the foam formulation such as varying the amount and type of polyol, catalyst, cross-linker, surfactant and blowing agent will result in different quality of foams. Several studies have been reported on the effect of diethanolamine [9], toluene diisocyanate index [8, 10] and stannous octoate [11] on the morphology, structure and physical properties of flexible foams. In this work, we studied the effect of water content as blowing agent on the mechanical properties, morphology, as well as thermal characters of the flexible foams.

A typical formulation was used to study the effect of isocyanate index on the physical properties of the flexible polyurethane foam (Table 1). The isocyanate index of every production was based on index 1.00 and the water content was altered by 2 pph, 3 pph and 4 pph of water.

Foam density can be controlled by regulating the water content [12]. The increase in water content in polyurethane formulation produced foams with lower density (Fig. 1). This is consistent with the work done by Lin and co-worker [13] by preparing water-blown flexible polyurethane foam with the addition of biomass materials. High density foams (above 50 kg/m^3) were prepared by 2 pph of water while low density foams (below 30 kg/m^3) were prepared by 4 pph of water. Hysteresis is a measurement of energy absorption in the foam. Foams with large energy absorption and dampening behaviour will exhibit high hysteresis measurement [14]. In other words, high hysteresis measurements produce foams with bad resilience and comfort to us-

Table 1. Standard formulation for flexible polyurethane foam.

	Component	Part (pph)
A	Palm Oil-based Polyester Polyol	50.00
	Commercial Polyether Polyol	50.00
	Tin Catalyst	0.05
	TEDA Catalyst	0.45
	DMEA Cross-linker	1.20
	Silicone Surfactant	1.50
	Blowing Agent (distilled water)	2.00
B	Toluene Diisocyanate	28.70
	Isocyanate Index	1.00

ers. Density can also affect foams' durability and support. Foam with high polymer density will typically retain its original properties to provide support and comfort of its origin design [15]. Foam hysteresis was inversely proportionate with the foam density. High density foam performed better hysteresis (H"23%), whereas low density foam performed bad hysteresis (H"58%).

Compression stress is one of the measurements for hardness in flexible cellular polymer [16]. The foam compression stress was increased with increasing water content used in the formulation (Fig. 2). When the amount of water content increases, the reaction of water with isocyanate leads not only to the formation of carbon dioxide for foam expansion, but also to the formation of urea hard segments [12]. These urea compounds can continue to react with isocyanate to form biurets, another hard compound in polyurethane. Therefore, the crosslinking hard segments in the cellular polymer are increased with the addition of water content to give a higher compression stress to the foam.

The measurements of strength and stiffness of cellular properties are important and among the basic parameters measured are tensile stress, elongation at break and tear resistance. There was a corelation among the tensile stress, elongation at break and tear resistance of the foam corresponding with the increment of water content in the foam formulation. Foams with higher tensile stress exhibited higher elongation property (Fig. 3) and tear resistance property (Fig. 4). The tear resistance property (0.2503 N/mm) of the foams was at a maximum with 3 pph of water content; but elongation at break (138.0%) and tensile stress properties (0.0970 Mpa) of foams were at a maximum with 2 pph of water content. Based on these evaluations, 2 pph of water content was chosen as the optimal water content as it produced foam with the lowest hysteresis and the highest elongation at break as well as tensile stress.

As the water content increased while the

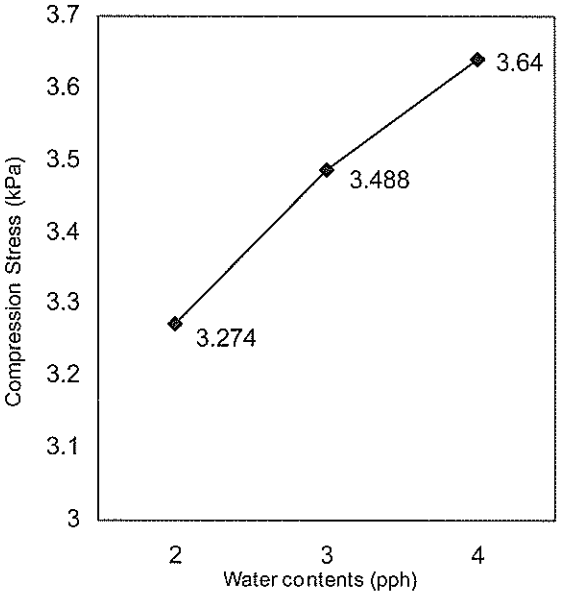


Figure 2. Effect of water contents on compression stress of the foam.

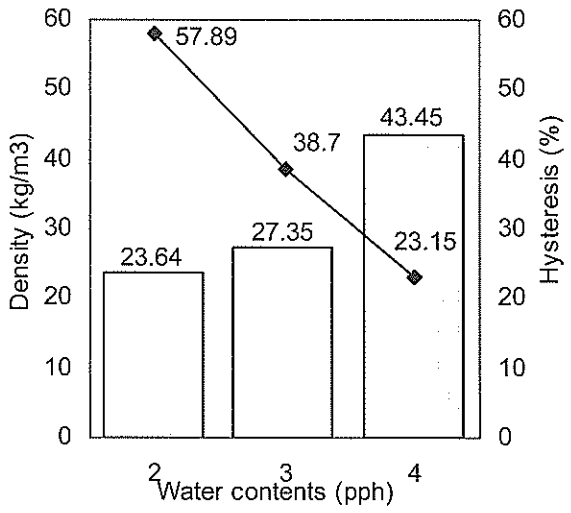


Figure 1. Effect of water contents on density and hysteresis of the foam. density, hysteresis.

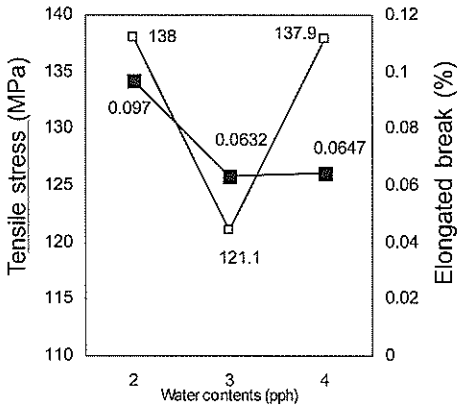


Figure 3. Effect of water contents on tensile stress and elongation at break of the foam. Tensile stress , Elongation at break.

isocyanate index was kept constant at 1.00, it was observed that the foams' open cell contents increased from 95 to 98% (Fig. 5). This may be attributed to the increment of carbon dioxide generation causing the blowing reaction to take place faster and subsequently producing higher open cell foams. Morphology from microphotograph analysis showed that foams prepared from higher amount of water contained bigger size cell in the structure (Fig. 6). However, at lower water content, cell structures were smaller and also more uniform [17].

The thermal characters of the foams altered by different water contents were recorded in Table 2. Although the temperatures of the melting peak (T_m) of the foams were in a narrow range between 575 and 583 °C, it still showed an increment corresponding with increasing water content in the foam formulation (Fig. 7). The decomposition temperature of foams produced from different water content from TGA pyrolysis also showed that the temperatures were increasing corresponding with increasing water content (Fig. 8). These phenomena were due to the additional formation of urea hard segments in the foam structure caused by the increase in water content. Therefore, it can be concluded that foams produced from higher water content were more stable thermally.

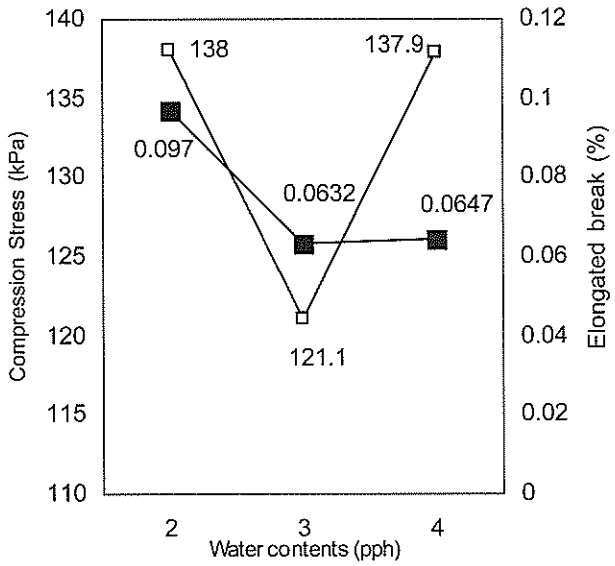


Figure 4. Effect of water contents on tensile stress and tear resistance of the foam. —■— Tensile stress, —□— Tear resistance.

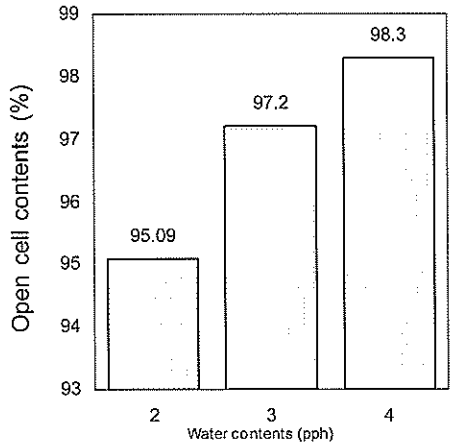


Figure 5. The effect of water contents on open cell contents of the foam.

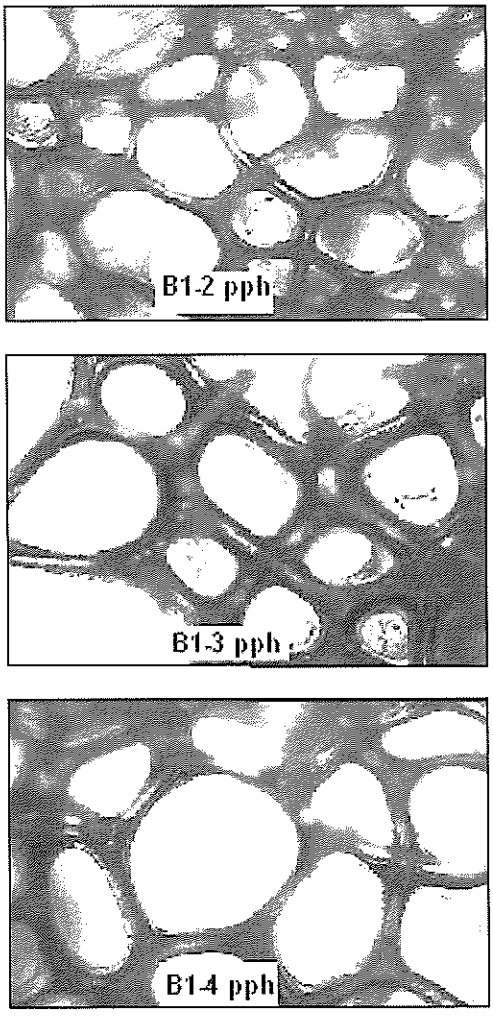


Figure 6. Microphotographs of open cell flexible polyurethane foam prepared from 2, 3 and 4 pph of water content at x40 magnification.

Table 1. Nutrient composition of cassava and sweetpotato compared with white rice (100 g dry weight basis).

Nutrient[1] Unit	(per100 g)	Cassava[2]	Sweetpotato[2]	White rice[3]
Proximates				
Water	g	12.0	12.0	12.89
Energy	kcal	349	333	360
Protein	g	2.97	6.08	6.61
Total fat	g	0.61	0.19	0.58
Ash	g	1.35	3.83	0.58
Carbohydrate, by difference	g	83.07	77.93	79.34
Dietary fibre, total	g	3.9	11.6	-
Minerals				
Calcium, Ca	mg	35	116	9
Iron, Fe	mg	0.59	2.36	0.80
Magnesium, Mg	mg	46	97	35
Phosphorus, P	mg	59	182	108
Potassium, K	mg	592	1305	86
Sodium, Na	mg	31	213	1
Zinc, Zn	mg	0.74	1.16	1.16
Copper, Cu	mg	0.218	0.585	0.110
Manganese, Mn	mg	0.838	0.999	1.100
Selenium, Se	mcg	1.5	2.3	-
Vitamins				
Vitamin C	mg	45.0	9.3	0.0
Thiamin	mg	0.190	0.302	0.070
Riboflavin	mg	0.105	0.236	0.048
Niacin	mg	1.864	2.157	1.600
Panthotenic acid	mg	0.234	3.099	1.342
Vitamin B-6	mg	0.192	0.810	0.145
Folate, total	mcg	59	43	9
Vitamin B-12	mcg	0.00	0.00	0.00
Vitamin A, IU	IU	28	54950	-
Vitamin A, RAE	mcg_RAE	2	2746	-
Vitamin E	mg	0.42	1.01	
Vitamin K	mcg	4.2	7.0	-
Amino acids				
Tryptophan	g	0.042	0.120	0.077
Threonine	g	0.061	0.322	0.236
Isoleucine	g	0.059	0.213	0.285
Leucine	g	0.085	0.356	0.546
Lysine	g	0.096	0.256	0.239
Methionine	g	0.024	0.112	0.155
Cystine	g	0.061	0.085	0.135
Phenylalanine	g	0.057	0.345	0.353
Tyrosine	g	0.037	0.132	0.221
Valine	g	0.076	0.333	0.403
Arginine	g	0.299	0.213	0.551
Histidine	g	0.044	0.120	0.155
Alanine	g	0.083	0.298	0.383
Aspartic acid	g	0.172	1.480	0.621
Glutamic acid	g	0.450	0.600	1.288
Glycine	g	0.061	0.244	0.301
Proline	g	0.072	0.201	0.311
Serine	g	0.072	0.341	0.347
Others				
ß-karotena	mcg	18	32957	-

[1]Nutrient value and weight are based on 100 g edible portion; [2]Converted to 12% moisture content from values for the fresh roots; [3]White (polished) rice, medium grain. *Source*: [2]

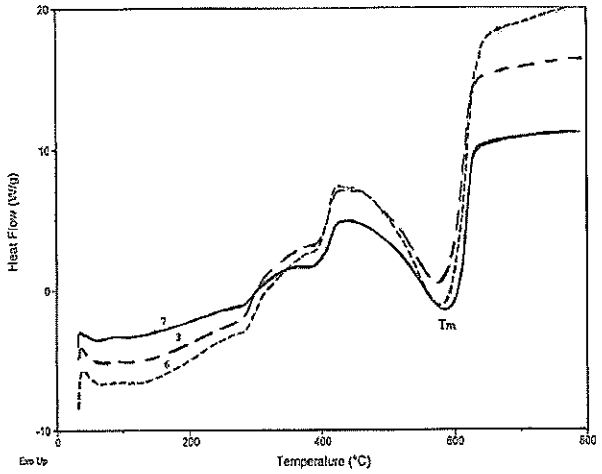


Figure 7. DSC curves of the polyurethane foams. Foam 3 - - - - produced by 2 pph water content; foam 6 ..... produced by 3 pph water content; foam 7 ——— produced by 4 pph water content.

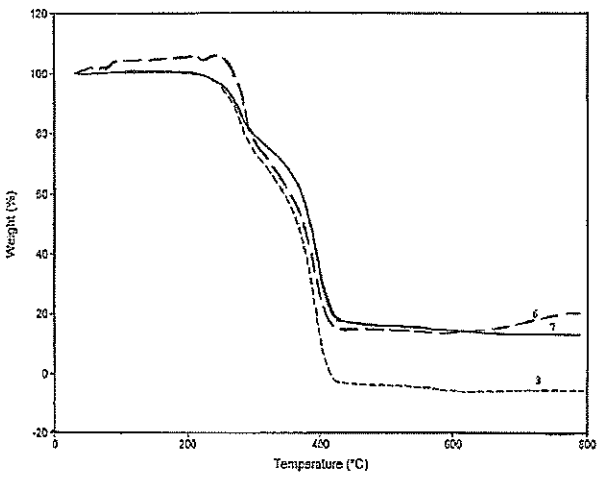


Figure 8. TGA curves of the polyurethane foams. Foam 3 ..... produced by 2 pph water content; foam 6 - - - - produced by 3 pph water content; foam 7 ——— produced by 4 pph water content.

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Hydrophilization of low density polyethylene by dielectric barrier discharge

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Abstract Dielectric barrier discharge (DBD) produced in atmospheric pressure air was used for the surface modification of low density polyethylene (PE) sample for the improvement in wettability. The effect of treatment time and applied power on the wettability of the sample was investigated by contact angle measurement. Contact angles of water and glycerol were used to determine the surface free energy of the sample. The results indicated that 1 second of exposure time was sufficient to make significant improvement in hydrophilicity of the sample. Wettability of the treated sample increased linearly with applied power. Stability of the modified surface was also investigated by measuring the contact angle up to several days after treatment. The results showed that the plasma modified surface recovered a large fraction of its original hydrophobicity within the first few days after treatment.

Keywords – dielectric barrier discharge – polyethylene – surface modification – wettability – hydrophilicity – contact angle – surface energy

INTRODUCTION

Most polymeric materials have chemically inert surfaces with low surface tension. This causes them to be non-perceptive to bonding with substrates, printing inks, coatings and adhesives. Polyethylene (PE) is an important engineering plastic due to its excellent mechanical properties and chemical stability. The hydrophobic nature of PE makes it excellent water repellent but its bondability and printability are poor. For a wide variety of applications of PE where hydrophilic surface is desired, surface modification is essential.

The wettability of polymer surfaces and its modification are becoming increasingly important for many applications. In the field of biomaterials, hydrophilic surfaces are highly desirable as the behaviour of a material in a biological environment is primarily determined by its interaction with water. Owing to that, considerable effort has been devoted to improve the wettability of polymers [1, 2].

The traditional methods of surface modification of fabrics and foils often include liquid chemical treatment, mechanical roughening and treatment by flame. These methods have poor uniformity and reproducibility. In comparison to these methods, dry plasma treatment forms an economical and environmentally friendly alternative. It is well known that plasma treatments can be used to modify the chemical composition of polymer surfaces so as to improve their surface property [3]. Most of the plasma sources operate at low pressure and demonstrate good treatment results. The disadvantage of these plasma systems are that vacuum equipment is expensive and continuous process is difficult [4].

Recently, much attention has been paid to the development of atmospheric pressure non-thermal plasma sources. The dielectric barrier discharge (DBD), as one of the main non-equilibrium plasma sources, can generate a non-equilibrium plasma under atmospheric pressure, requiring no expensive vacuum equipment, which is a must for conventional

low-pressure glow discharge, and so processing cost is reduced and the ability of continuous processing is improved, and therefore large-scale industrial applications are possible [5-7]. Because of the thermal non-equilibrium between electrons and the bulk of the plasma, many physico-chemical reactions of interest can be carried out at near ambient temperature without any damage to the bulk of the material.

The subject of our study is the application of DBD for the surface modification of PE with special reference to wettability. In our experiment we have indigenously fabricated a DBD system which could be used for a wide range of industrial application.

EXPERIMENTAL DESCRIPTION

Treatment of the sample

PE film of commercial variety of surface density of 20-30 g/m^2 was used for the study. The films were washed in ultrasonic bath with isopropyl alcohol for 10 minutes and then dried in air. The plasma processing system consisted of a dielectric barrier unit operated by a high voltage high frequency power supply (0-20 kV, 10-30 kHz). The high voltage was applied to two cylindrical metal electrodes with dielectric barrier surrounding it. Glass of thickness 1 mm was used as barrier to the electrodes. The gap between the barrier electrodes could be adjusted from 2-4 mm. The power delivered between the parallel electrodes was 35W and 50W. The electrical characterization of the discharge was carried out by a high frequency digital oscilloscope TDS 2002. The scheme of the treatment process of PE film in the discharge is shown in figure 1. The sample to be treated was passed through the discharge at a uniform speed so that it was possible to determine the exposition time. The exposure time for the plasma processing was varied from 1 to 8 seconds.

Surface characterization of the plasma treated sample

The effect of the treatment time and applied power on the surface property of the sample was investigated by measuring the contact angle of untreated and plasma treated PE with water and glycerol. The experimental arrangement for contact angle measurement is shown in figure 2. The system offers a computer aided contact angle and surface energy analysis facility. The average contact angle was calculated from the measurements on at least three drops of liquid placed on different parts of the sample, each drop measured two times at diametrically opposite sides. Contact angles of the samples were measured immediately after the treatment except in the case of analysis of stability of the modified surface. In this case contact angles were measured at different time intervals after treatment. The surface free energy of the PE sample was determined from the contact angles of water and glycerol with the PE surface using Owens-Wendt-Kaelble two liquid method [8-10]

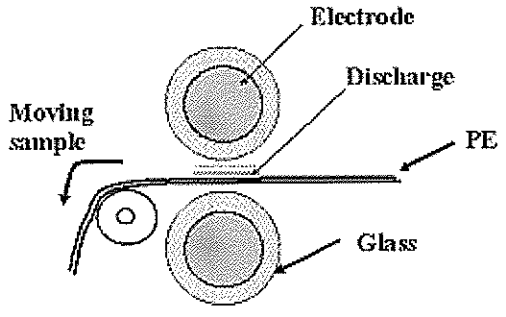

Fig. 1 Schematic diagram of the treatment PE in a DBD

Figure 1. Schematic diagram of the treatment of PE in a DBD

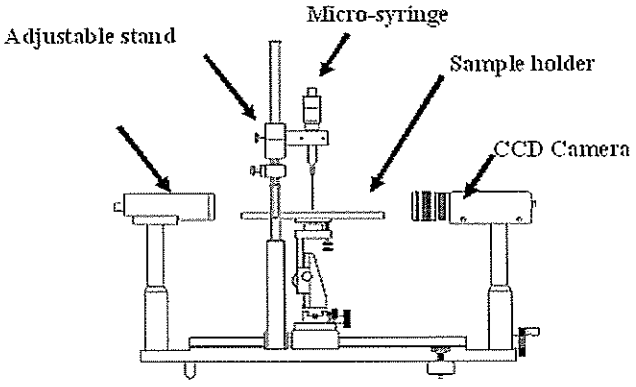

Figure 2. Experimental setup for the measurement of contact angle.

$$\gamma_l(1+\cos\theta) = 2(\gamma_l^d\gamma_s^d)^{1/2} + 2(\gamma_l^p\gamma_s^p)^{1/2} \quad (1)$$

where γ_l^d and γ_l^p are dispersion and polar components of the surface tension of the probe liquid respectively, and γ^d, γ^p are dispersion and polar components of the surface free energy of the solid respectively. The total surface free energy of the liquid is γ_l and the contact angle between the sample and liquid is θ. The total surface free energy of the solid is equal to the sum of the polar and dispersion components.

RESULTS AND DISCUSSION

Effect of treatment time

The change in wettability of PE surface after various treatment time in DBD was quantitatively investigated by measuring contact angles with water and glycerol. Images of water drop on the surface of untreated and plasma treated PE surface are depicted in figure 3. The water contact angle on PE after treatment in DBD at power 50 W is plotted as a function of treatment-time in figure 4. The contact angle decreased rapidly from the original value of 88° to about 45° already after 1 s of treatment. Figure 5 shows the corresponding total surface free energy as a function of treatment time. Significant increase in the total surface free energy with the treatment time was observed even for the first second of treatment. For longer treatment time the surface free energy exhibited saturation, a sign of reaching equilibrium between the surface modification and removal of the modified layer by etching process. The values of total surface free energy (γ), its polar component (γ^p) and the dispersion component (γ^d) for PE are plotted in figure 6. It is also interesting to note that the polar component of surface free energy was mainly responsible for the increase in the total surface free energy. There was no significant change in the value of the dispersion part of the surface free energy. These results are in agreement with the other results in our previous works concerning the plasma surface modification of polymers [11-13]. The enhanced polar component of surface energy directly corresponds to the incorporation of polar or hydrophilic functional groups such as: carbonyl, hydroxyl and carboxyl groups.

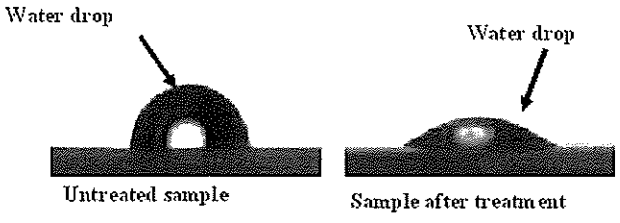

Figure 3. Images of water drop on an untreated and plasma treated PE surface.

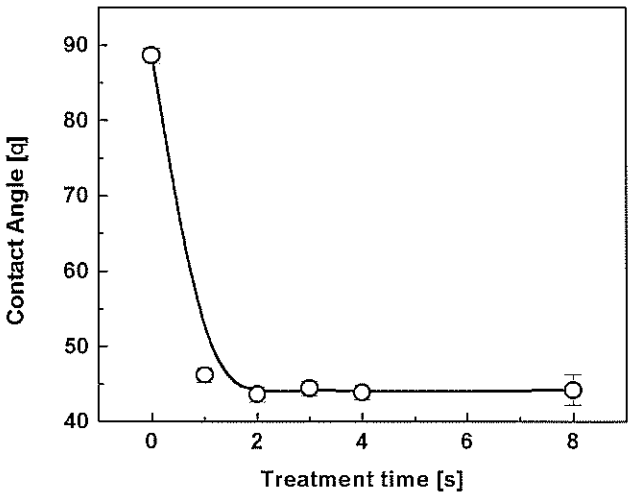

Figure 4. Water contact angle on PE as a function of treatment time in DBD. The applied power was 50 W.

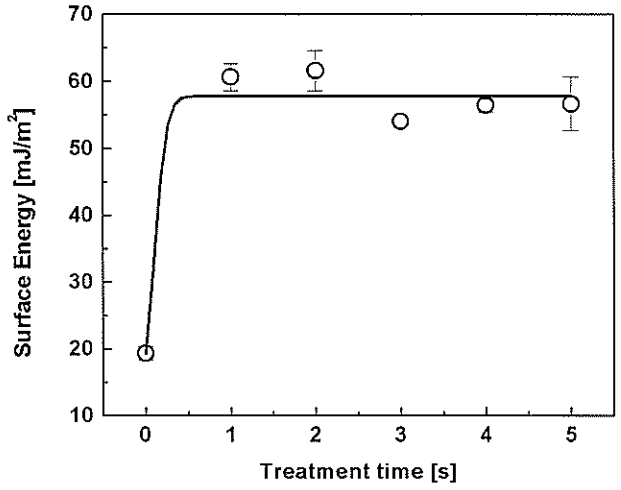

Figure 5. Surface free energy of PE as a function of treatment time in DBD. The applied power was 50 W.

Effect of applied power

The plot of contact angle as function of applied power is shown in figure 7. The results show that the value of contact angle decreased proportionally with increase in applied power. The corresponding surface free energy of PE as function of applied power is plotted in figure 8. The surface free energy increased linearly with the applied power. The increase in surface free energy of the treated sample was remarkable since a small applied power (35 *W*) was sufficient to change the surface energy of the sample from about 20 *mJ*/m^2 to about 50 *mJ*/m^2. The energy of the plasma species i.e. ions, electrons and photons was proportional to applied power density. The higher the applied power the greater the energy with which the particle bombard the surface of the material under treatment. Moreover, the flux of charged particles also increased with the increase in applied power. This led to the strong dependence of the change in surface property with the applied power.

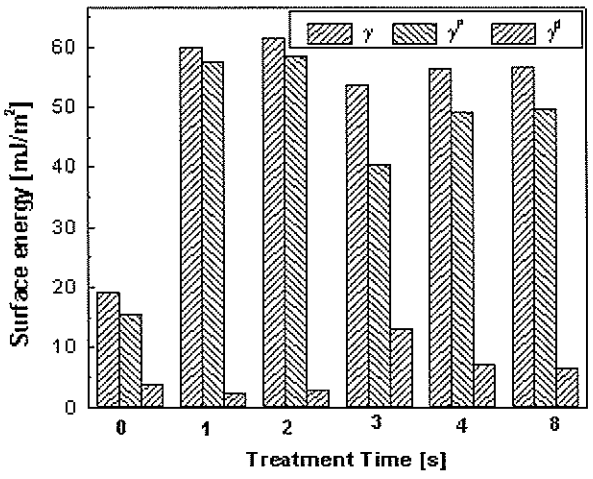

Figure 6. Surface free energy along with its polar and dispersion components as functions

Stability of the modified surface

The other part of the present work consisted of the investigation of the stability (aging effect) of the hydrophilic property of the plasma modified surface. It was carried out by measuring the contact angle at different time intervals after treatment by storing the sample in dust free environment.

The contact angle as function of time after treatment is shown in figure 9. The results show that the hydrophilicity of the treated sample degraded quickly at first and then slowly with storage time. However the sample recovered a significant fraction of hydrophobicity during the first 24 hours of storage time. In general, the causes of aging could be attributed to thermodynamically driven reorientation of polar species away from the surface into subsurface. This effect also arises due to the absorption of polar and non-polar contaminants, the diffusion of atoms and the loss of oxygen to the atmosphere from the treated layer of the polymer

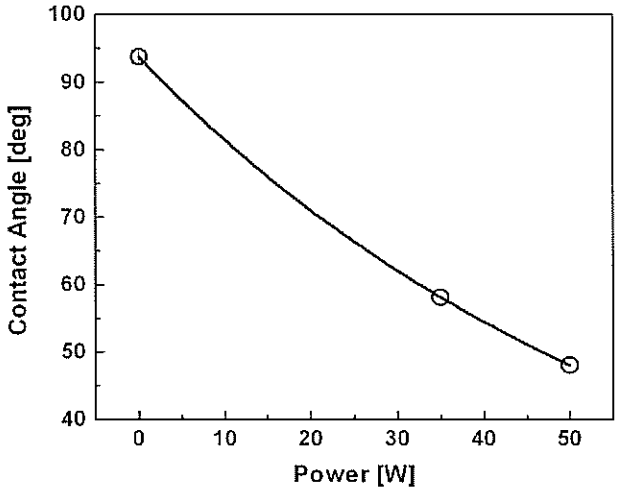

Figure 7. Water contact angle on PE as a function of applied power in DBD. The treatment time was 8s

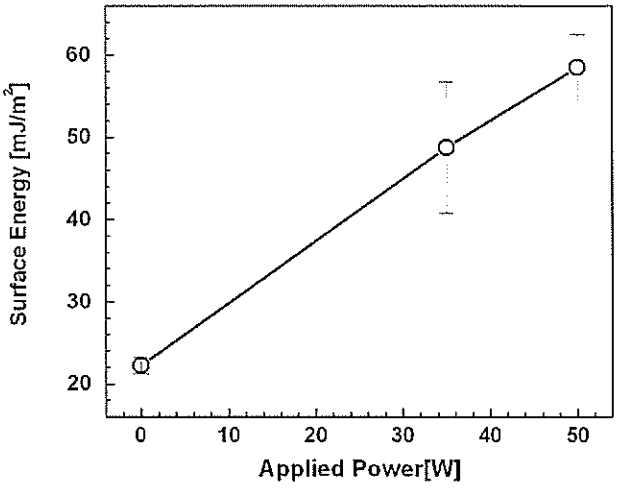

Figure 8. Surface free energy of PE surface as a function of applied power in DBD. Thtreatment time was 8s.

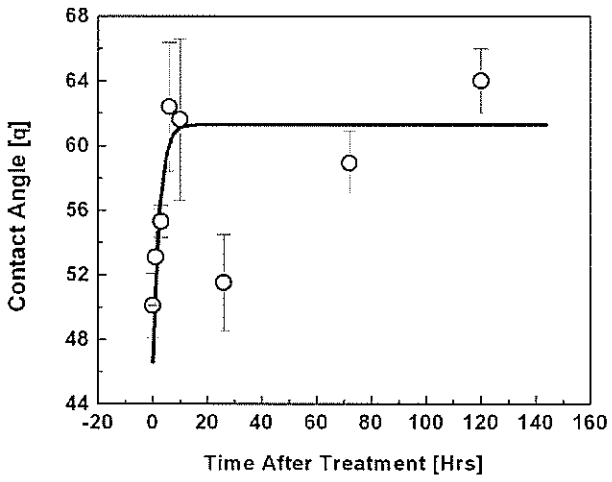

Figure 9. Water contact angle on PE as a function of storage time after treatment (aging time).

surface [1]. Although plasma surface modification is an efficient technique for treatment, its stability is limited due to the presence of aging effect with typical evolution of contact angle of a treated polymer.

Conclusion

The application of the dielectric barrier discharge (DBD) to improve the wettability of PE film has been tested successfully in this laboratory. It was found that the hydrophilicity of the PE film surface is linearly dependant on discharge power, while the effective exposure time can be as brief as 1 second. In order to understand the plasma processes leading to these effects, it is necessary to determine the condition of the plasma produced in the DBD. Experiments to measure the electron temperature and density by Langmuir probe and emission spectroscopic techniques will be carried out in this laboratory in the near future.

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Table 2. Glycaemic indices (GI) for white rice, cassava and sweetpotato.

Food	Glycaemic index
White rice	70
Cassava	46
Sweetpotato	50
White bread	96
Glucose	100

Source: [3]

gested food is converted to glucose in the blood, with glucose having a GI of 100 and white bread of 96. This reinforces the fact that cassava and sweetpotato are more suitable foods for diabetics.

VERSALITY OF SWEETPOTATO

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is a member of Convolvulaceae, or the morning glory family. The centre of origin of sweetpotato is in the Andean mountains of Peru and Colombia [4]. New Guinea is also postulated as a secondary centre of diversity, with evidence showing that the species had reached the highlands 1200 years ago. More than 5000 cultivars were discovered in these isolated ecological conditions, growing (uncharacteristically) at altitudes up to 2800 m. The cultivars from Papua New Guinea have been found to be substantially different from germplasm in South America [5].

Thus, it is not surprising that sweetpotato has a cultivation range from 40°N to 32°S [6], and is planted in Japan and Korea down to New Zealand, covering temperate as well as tropical regions, in both the developing and developed countries. The world's largest producer of sweetpotato is China with 107,176,100 tonnes per year and accounting for 83% of total production. Uganda (with 2,650,000 tonnes) and Nigeria (2,516,000 tonnes) rank as a poor second and third [7]. While not strictly a primary staple (except in parts of Polynesia), sweetpotato is eaten as a supplementary food.

Sweetpotato is prepared in a variety of ways depending on the wide ranging cultures of those who plant it. The simplest methods are by boiling, steaming, baking and frying (with or without batter). In USA, sweetpotato features prominently on Thanksgiving Day in the southern states during which sweetpotato pie is invariably served. In Japan, where there has been a long tradition of eating sweetpotato, it features weekly in most homes as one of the ingredients in *tempura*. The Japanese love affair with sweetpotato has bloomed in many creative ways in using it – in bread, cakes, biscuits, noodles, jam and spreads, ice cream, confectionery, snacks, straight and mixed juices, yoghurt drinks [8], to name some.

Although sweetpotato shoots and leaves have a much higher protein content (28-32% dry matter basis) than the roots [9], they are only eaten in South-east Asia. Just like *kangkong* (water convolvulus or water spinach, *Ipomoea reptans* = *I. aquatica*), sweetpotato shoots can serve as a green vegetable, and be cooked in a similar fashion, e.g. stir-fried with ground chilli and *belacan* (shrimp paste) or with fermented beancurd. In Japan and Korea, only the petioles of sweetpotato are eaten. Research in Japan has shown high phytochemical activity in sweetpotato leaves which can benefit human health [10, 11]. In most countries, shoots and leaves are considered as crop residues, and at best are sometimes fed to ruminant livestock [12].

The world's germplasm collection of this crop maintained at the International Potato Center (CIP) in Peru totals 5702 accessions, of which 1197 are classified as wild [13].

PRODUCTION TECHNOLOGIES FOR ROOT CROPS

Research on cassava and sweetpotato at MARDI has been going on since the 1970s, and complete technology packages for planting these crops in Malaysia have been published in two manuals: *Manual Teknologi Penanaman Ubi Kayu* and *Manual Teknologi Penanaman Ubi Keledek.*

Both these root crops adapt well to the humid tropics like in Malaysia. However, sweetpotato has several advantages over cassava:

1. It can grow well on marginal soils, such as *bris*, tin-tailings, acid sulphate soils and drained peat, if the recommended soil

Table 3. Root yield of sweetpotato cv. 'Gendut' on various soil types.

Location	Soil type	Root yield (t/ha)	Remarks
Serdang[1]	upland mineral	17.4	Yield trial in research station
Seberang Perai[1]	upland mineral	22.3	Yield trial in research station
Kg. Bindu[2]	sand-tailings	20.3	Local verification trial on farmer's field
Bidor[2]	sand-tailings	26.2	Local verification trial on farmer's field
Sepang[2]	drained peat	20.3	Local verification trial on farmer's field
Pontian[2]	drained peat	20.4	Yield trial in research station
Kandis[2]	*bris*	30.0	Local verification trial on farmer's field
Rhu Tapai[3]	*bris*	26.4	Yield trial in research station
Kuala Linggi[4]	acid sulphate soils	23.4	Yield trial in research station

Sources: 1 [14]; 2 [S. L. Tan, unpublished]; 3 [15]; 4 [16]

amendments are adopted (as exemplified by the yield performance of the cultivar Gendut in Table 3). This means that sweetpotato will not be competing with other crops for fertile and better soils. Table 4 shows the extent of marginal soils in Peninsular Malaysia.

2. Being a ground-hugging crop, sweetpotato can be grown in areas with strong winds unlike cassava which lodges easily in windy places, causing yield losses.
3. Sweetpotato is harvested after 3½-4 months (which translates to a faster rate of returns), whereas the edible varieties of cassava are harvested after 8-10 months.
4. Table 1 has already shown that the nutritive value of sweetpotato is far superior to that of cassava.

MARDI has successfully developed a number of sweetpotato cultivars with improved root yields and/or quality (Table 5).

WAYS OF CONSUMING SWEETPOTATO

From the points of view of its nutritional value and adaptability to planting in Malaysia, sweetpotato can take on the role as a supplementary staple so that we do not have to depend entirely on rice. To encourage people to eat sweetpotato, there are many ways of preparing it other than just boiling, steaming, baking or frying.

Most people are unaware that we are also dependent on wheat as a secondary (but less important) staple. This is easily proven: many of us have in our daily diet bread, buns, cakes, biscuits, noodles, *roti canai* and *chappati*, all of which are made from wheat flour. Annual imports of wheat amount to around RM800 million, and this figure has probably gone up with the recent increases of wheat price in the world market (just like in the case of rice).

The good news is that flour can be made from sweetpotato to replace a portion of the wheat flour

Table 4. Extent of marginal soils suitable for growing sweetpotato in Peninsular Malaysia.

Soil type	Extent (ha)	Notes
Sand tailings	91,000	A dominant fraction (80%) of ex tin-mining land.
Bris soils	165,000	Fairly widespread along the East Coast of Peninsular Malaysia; some currently under tobacco.
Acid sulphate soils	110,000	Severe acidification of soils of marine floodplains upon drainage and aeration due to oxidation of mainly pyrites. Found in Kedah, Melaka, Johor and Terengganu.
Organic soils	870,000	Mainly peat and some muck.
Idle paddyland*	433,000	Abandoned, or in the off-season.

*Not generally favourable, but can be used if sweetpotato is planted on large beds