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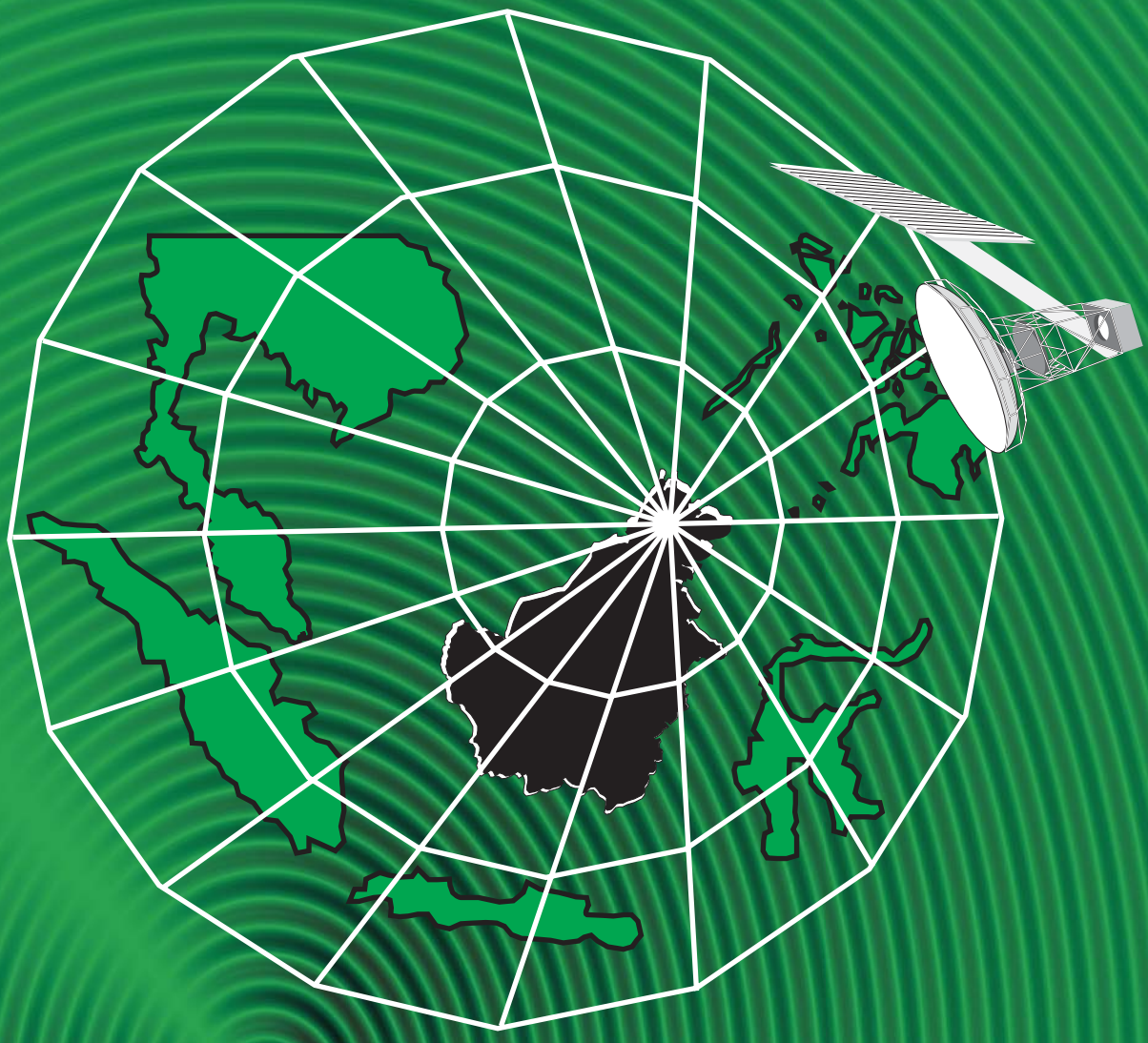
JURNAL SAINS DAN TEKNOLOGI



BORNEO SCIENCE

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Volume 23 **September 2008**

CONTENT
KANDUNGAN

Page
Muka Surat

ORIGINAL ARTICLES

RESEARCH NOTE

DIRECTED FISHERIES FOR DOLPHINS AND DUGONG IN SABAH, EAST MALAYSIA: PAST AND PRESENT

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ABSTRACT. *An interview survey was used to determine the nature and magnitude of directed catches of marine mammals and to estimate the associated level of its mortality in Sabah, East Malaysia. Between March 1997 and December 2004, we interviewed 1,186 fishermen, village headmen and/or knowledgeable villagers along the coastline. They were asked questions about sightings of marine mammals and past and present utilisation. A total of 294 (25%) interviewees said they have hunted the animals or reported hunting activities of their fathers or grandfathers in the past. Of this, 231 (79%) hunters caught dugongs (Dugong dugon), 14 (5%) hunted dolphins (Tursiops spp., Stenella spp.) and 49 (17%) hunted both groups of animals. The magnitude of dugong catches in the past was similar throughout Sabah, but significantly greater for dolphins in the Sulawesi and Sulu Seas than in the South China Sea. Majority of hunters are fishermen using pump boats and/or gillnets. Harpoon or spear is the main hunting gear. About 326 dolphins and 796 dugongs were reported to be taken annually with an average catch of 5.2 dolphins (95% CI = 4.01, 6.34) and 2.8 dugongs (95% CI = 2.47, 3.21) per hunter. The bootstrapped estimates of dolphins and dugongs taken annually in each region and for each boat-type were extremely high and unsustainable. Most hunters had stopped hunting in the 1980's and only 32 (11%) said they still hunt dolphins or dugong, at least occasionally or opportunistically during fishing trips. A dedicated monitoring and educational program is urgently required to significantly reduce the threat.*

KEYWORDS. Conservation; dolphin; Dugong dugon; hunting; Sabah; utilisation.

INTRODUCTION

East Malaysia, which comprises the states of Sabah and Sarawak, and the Federal Territory of Labuan, occupies the northern one-third of the island of Borneo (Figure 1). Sabah is the second largest state in Malaysia with an estimated land area of 73,600 km² (DSM, 2001). It has, however, the longest coastline of any Malaysian state, of approximately 1,600 km (TRPDS, 1998). The state had a population of approximately 2.6 million in 2000, not counting the substantial number of illegal immigrants from Indonesia and the Philippines (DSM, 2001). It is estimated that considerably more than 75% of the population live and work in the coastal area.

The majority of Sabah coastal communities are from the ethnic Bajau, who consist of several groups of people, such as the Bajau Laut, Bajau Pelauh and Bajau Ubian. Other ethnic groups are the Bugis, Sungai, Kedayan and Brunei. Most of them are artisanal fishermen who largely depend on the seas or rivers, and their surroundings, for food and to make a living. Many employ gillnets and traditional fishing gears, such as fish stakes and portable traps, hook-and-line, bag nets, lift nets, barrier nets and scoop nets, and use small non-powered or outboard-powered boats (DFS, 2003).

Besides the dugong, there are at least 17 species of cetaceans (two species of Mysticeti and 15 Odontoceti) that have been confirmed to occur in Sabah waters (Jaaman, 2001; 2004). The most common species found in coastal waters, especially in major bays and estuaries, are the Irrawaddy dolphin *(Orcaella brevirostris)* and Indo-Pacific humpbacked dolphin *(Sousa chinensis)* (Beasley and Jefferson, 1997; Jaaman *et al.*, 2001; Jaaman, 2001; 2004). In addition, the Indo-Pacific bottlenose dolphin *(Tursiops aduncus)*, spinner dolphin *(Stenella longirostris)* and pantropical spotted dolphin *(Stenella attenuata)* have been reported as the most abundant cetaceans in the open waters of East Malaysia (Beasley, 1998; Jaaman, 2001; 2004). The dugong was the most common marine mammal species recorded stranded between 1996 and 2001 (Lah-Anyi and Jaaman, 2002).

These marine mammals are familiar to and have coexisted with Sabah fishing communities for centuries. However, dolphins and dugongs are also killed in some locations, both incidentally in fisheries targeting other species and deliberately for human consumption and/or for use in cultural or traditional ceremonies (Jaaman *et al.*, 1999; 2000; Jaaman and Lah-Anyi, 2002, 2003; Jaaman, 2004). In Malaysia, directed fisheries for dolphins and dugong are only known to occur in Sabah (Jaaman and Lah-Anyi, 2003; Jaaman, 2004). Despite federal and state legislation that protect the species, this activity is apparently not being monitored or documented.

This study represents a first attempt to determine the nature and magnitude of directed catches of marine mammals and estimate the associated level of mortality from hunting in Sabah by using interview surveys. Site visits and interviews have been used to collect information on marine mammal catches in Canada, Spain, Philippines, Thailand, Vietnam, Cambodia, Indonesia, Palau, Solomon Islands and the Torres Strait between Australia and Papua New Guinea (Barnes, 1991; 2005; Dolar *et al.*, 1994; 1997; Lien *et al.*, 1994; Marsh *et al.*, 1995; 1997; 2002; Perrin *et al.* 1996; 2005; Persoon *et al.*, 1996; Takekawa, 2000; López *et al.*, 2003).

MATERIALS AND METHODS

Study area

The study area is the coastline of Sabah, which is surrounded by the South China Sea and the Palawan Thrust to the west, the Sulu Sea to the northeast and the Sulawesi Sea to the east. Overall, there are three fishing regions and 16 fishing districts (Figure 1, Table 1). The fisheries are predominantly coastal, with more than 70% of the catches taking place within 30 nautical miles (nm) from shore (DFS, 2003). According to the Summary of Annual Fisheries Statistics Sabah 2002 (DFS, 2004), the total landings from the marine fishery sector were 175,122 metric tons (mt) with a wholesale value of about RM584 million (US$154 million). Commercial (gillnets, trawl nets and purse seines) and traditional gears contributed 130,331 (74%) and 44,792 (26%) mt, respectively. Based on a listing made in 1998, there were 20,845 registered fishermen and the fishing fleet consisted of 10,456 boats (Table 1).

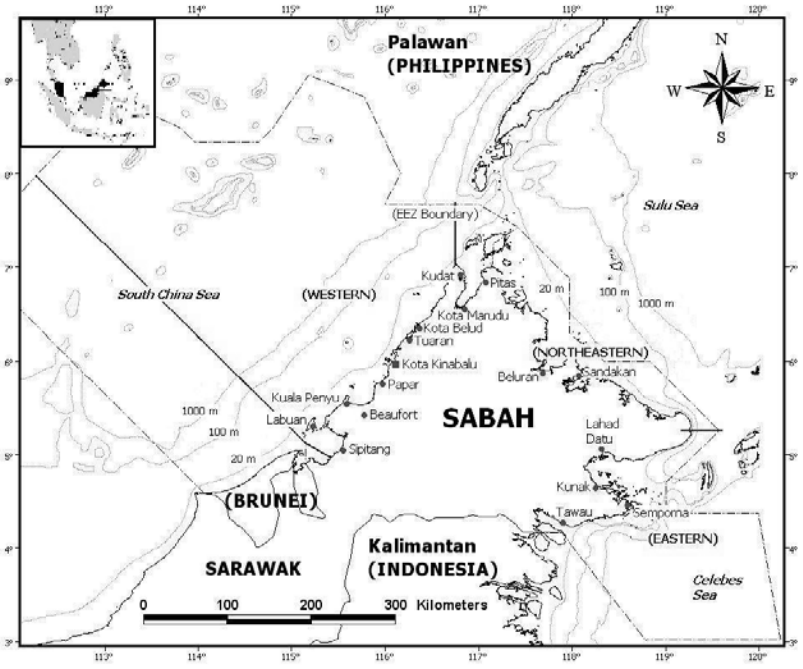

Figure 1. Sabah, with location of fishing districts/landing points (towns) and the survey regions.

Data collection

Semi-structured and informal interviews (based on Dolar *et al.*, 1994; 1997; Aragones *et al.*, 1997) were used. Between March 1997 and December 2004, fishing villages, fish markets, fish landing jetties and anchored fishing boats in all fishing districts in the study area were visited. Interviews were conducted as part of a wider study on marine mammal interaction with fisheries in East Malaysia.

During site visits, fishermen, village headmen and/or knowledgeable villagers were asked questions about sightings of marine mammals, the incidence and frequency of hunting and the species involved (Table 1). In addition, their awareness of the government regulations on fisheries was also noted. To assess the reliability of the respondents and their answers, several test (validation) questions were asked (i.e. to which a respondent would be expected to know the answer and to which the answer is not known). Officers from relevant local authorities (Department of Wildlife Sabah, Department of Fisheries Sabah, Sabah Parks) who had extensive knowledge of the community, area, and local fishing industry assisted in conducting the interviews.

Any indication of marine mammal hunting activity in the area was photographed. Respondents' independent reviews of illustrations in field guides (Leatherwood and Reeves, 1983; Leatherwood *et al.*, 1988; Jefferson *et al.*, 1993; Tan, 1997) and a poster called *Mamalia Marin Malaysia* produced by the Universiti Malaysia Sabah were used to determine the species of marine mammal taken and present in the area.

Table 1. Fishing regions, districts, landings and register of fishermen and boats in Sabah (based on DFS, 2004).

Region/District/Landings	Gear-type	Number of fishermen[a]	Number of boats[a]
Western (South China Sea) / 7 districts – Kota Belud, Tuaran, Kota Kinabalu, Papar, Beaufort, Kuala Penyu and Sipitang / 78583 metric tons	Traditional	2537	887
	Gillnets	1852	1743
	Trawl nets	970	246
	Purse seines	366	88
	All gears	5725	2964
Northeastern (Sulu Sea) / 5 districts – Sandakan, Beluran, Pitas, Kota Marudu and Kudat / 44634 metric tons	Traditional	3119	1915
	Gillnets	3147	1981
	Trawl nets	3297	993
	Purse seines	270	26
	All gears	9833	4915
Eastern (Sulawesi Sea) / 4 districts – Tawau, Semporna, Kunak and Lahad Datu / 51905 metric tons	Traditional	2648	1654
	Gillnets	942	632
	Trawl nets	856	183
	Purse seines	841	108
	All gears	5287	2577
All regions / 16 districts / 175122 metric tons	Traditional	8304	4456
	Gillnets	5941	4356
	Trawl nets	5123	1422
	Purse seines	1477	222
	Grand Total	**20845**	**10456**

[a] Listing made in 1998

Analysis of hunting rates

Interview data were analysed to estimate a "minimum" hunting rate. Data were divided into strata on the basis of fishing region and boat-type. Fishing boats were categorised as non-powered, outboard-engine, pump-engine and inboard-engine boats. A total of 1,186 respondents was interviewed. The respondents are assumed to be representative in each category, i.e. the proportion of respondents reporting catches and the calculated hunting rates can be raised to give estimates for catches by all fishermen in the state.

The basic question asked to respondents reporting catches was how many marine mammals were taken by them each month or year. Not all answers were fully quantitative and some respondents gave qualitative answers, such as "a few" or "some" or "many". These respondents were then asked to give the number of animals versus the number of months/years in a range. When answers encompassed a range of values, such as 1 per 2-3 months, 1 per 6 months, 2-5 per year, 5-10 per year, 10-20 per 5 years, or 10-20 per 10 years, the mid-point value was taken and all estimated hunting rates were standardised into the number of animals taken per year. For answers such as > 10 per year, > 20 per 5 years, or > 20 per 10 years, the minimum figure was taken and divided by the number of years.

The overall mean annual catch per hunter for each region and boat-type is given by the total number of animals taken per year divided by the number of fishermen reporting animal catches. Separate totals were estimated for dolphins and dugongs. The total number of animals taken annually is estimated using the number of fishermen in each region and boat-type, reported in the Summary of Annual Fisheries Statistics Sabah 2002 (DFS, 2004), as a raising factor. To calculate summary statistics, data from respondents using outboard- and pump-engine boats were combined in the outboard-powered category (since that the Summary of Annual Fisheries Statistics Sabah 2002 only gave figures for non-powered, outboard- and inboard-powered categories).

Analysis of factors affecting the reported incidence of hunting of marine mammals was based on Generalised Linear Models (GLM), fitted using BRODGAR software (Highland Statistics Ltd.). The response variables were the presence (1) or absence (0) of hunting in the past of (a) all marine mammals, (b) cetaceans or (c) dugongs. Explanatory variables considered were: interview year, region, ethnic origin, fishing gear-type and boat-type, which were all nominal variables. The models were run assuming a binomial distribution for the response variable and using a logit link function.

The initial models had the formula:

$$(Y1) \sim \alpha + \text{as.factor (interview year)} + \text{as.factor (region)} + \text{as.factor (ethnic origin)} + \text{as.factor (fishing gear type)} + \text{as.factor (boat-type)} + \varepsilon_i$$

Where; Y1 is the occurrence of hunting, α is the intercept, ε_i is the residual (unexplained information or noise, $\varepsilon_i \sim N(0, \sigma^2)$). Nominal explanatory variables were recoded as binomial dummy variables. For example, the regions analysed were Western, Northeastern and Eastern. Two dummy variables were thus created, for Northeastern and Eastern, and a significant coefficient value indicates a difference from Western. In each case, the final (best-fit) model was identified using stepwise removal of non-significant terms until no further decrease in the Akaike Information Criterion (AIC) value was seen. The individual probability (P) value associated with each explanatory variable in the final model was used to identify significant effects on the occurrence of hunting. Binomial GLM was also used to determine the factors affecting respondents' awareness (either aware or not aware) of the government regulations on wildlife and fisheries.

Similar analyses were carried out on the variation in numbers of cetaceans and dugongs reported to be killed. The incidence of hunting was rare and can be modelled with a Poisson distribution. However, the numbers of animals reported caught per year varied widely, ranging from 0.2 to 18 (i.e. the distributions were "over-dispersed"). In this case, a quasi-Poisson distribution that includes a dispersion parameter, and log link function was assumed for the response variables.

Confidence limits for the total number of marine mammals caught annually by fishermen were estimated using a bootstrap procedure. A purpose-written BASIC programme was used to simulate the data collection procedure, repeatedly re-sampling with replacement from the set of N interviews in a stratum to generate multiple sets of N interviews. In the present application 10,000 repeats were used, each yielding an estimate of the number of animals taken, raised to the level for all fishermen in the region. In each case, the 10,000 estimates were then sorted and the 251st and 9,750th values represent the 95% confidence limits (i.e. only 5% of values are more extreme). Interviews were stratified by fishing region and boat-type and confidence limits derived separately for each region and boat-type. Confidence limits were also derived for the total across all regions and all boat-types, by running a version of the programme in which all strata were sampled, the total number of animals taken stored, and the procedure repeated 10,000 times.

RESULTS

Hunting rates estimated from interview data

Of 1,186 respondents interviewed, 294 (25%) said they hunted marine mammals or reported hunting activities of their fathers/grandfathers in the past (Table 2). Two hundred and thirty-one (79%) caught dugongs, 14 (5%) caught dolphins and 49 (17%) caught both groups of marine mammals. Hunting was reported in all interview year, interview season, region, ethnic origin, fishing gear-type and boat-type categories, except by fishermen using inboard-powered boats.

An estimated total of 326 dolphins and 796 dugongs was reported taken annually with a mean catch of 5.2 dolphins (95% CI = 4.01 – 6.34) and 2.8 dugongs (95% CI = 2.47 – 3.21) per hunter (Table 2). The Northeastern region recorded the highest number of dolphin or dugong hunters, number of animals caught and mean annual catch per hunter. The pump-engine boat category recorded the highest number of dolphin hunters, number of animals caught and mean annual catch per hunter. Although the numbers of dugong hunters and animals caught were highest in the pump-engine boat category, the highest mean annual dugong catch per hunter was recorded in the outboard-engine boat category.

Around 4,626 dolphins (95% CI = 3,267 – 6,185) and 12,279 dugongs (95% CI = 10,554 – 14,103) were estimated to be caught annually by fishermen in the past (Table 3). About half of the estimated total numbers of dolphins and dugongs caught were from the Northeastern region. The combined outboard-powered boat category contributed the majority of the total estimated catch.

Factors affecting the reported hunting incidence

In conducting GLM analysis, data from fishermen using inboard-powered boats were excluded as this category reported no marine mammal hunting (i.e. all values for numbers of animals caught were zero). All fishermen using non-powered boats reported zero dolphin catches and these data were excluded from the dolphin hunting analysis.

Binomial GLM confirmed the existence of significant effects of fishing gear-type and boat-type on the overall reported incidence of marine mammal hunting in the past (Table 4). A higher proportion of fishermen using gillnets admitted to hunting marine mammals, as compared to fishermen using traditional gears. A higher proportion of fishermen using non-powered boats said they hunted in the past, as compared to fishermen using boats with outboard- and pump-engines. There were no effects of interview year, region and ethnic group.

In the case of dolphin hunting, all fishermen who admitted hunting were from the ethnic Bajau and this variable was subsequently excluded from the model. Only region had significant effect on the reported incidence of dolphin hunting. A higher proportion of respondents from the Northeastern and Eastern regions said they hunted the animals, as compared to respondents from the Western region.

In the case of dugong hunting, there were significant fishing gear-type and boat-type effects. As for hunting of marine mammals in the past, there was a higher incidence of dugong hunting reported from fishermen using gillnets than traditional gears. A higher proportion of fishermen using non-powered boats said they hunted dugong in the past, as compared to fishermen using boats with outboard- and pump-engines.

The quasi-Poisson GLM for numbers of dolphins reported killed included effects of interview year, region and boat-type, but only the effect of region and boat-type were significant (Table 5). Numbers reported killed were higher for the Northeastern and Eastern regions than the Western region and higher for fishermen using pump-engine boats than boats with outboard-engines. There was no effect of ethnic group. The quasi-Poisson GLM for numbers of dugongs reported killed included no significant effect of any of the explanatory variables.

Table 2. Summary of marine mammal hunting in Sabah; showing the total number of fishermen, number of fishermen interviewed, number of fishermen reporting animal catches and the mean annual catch per hunter.

		INTERVIEWS			DOLPHIN HUNTING					DUGONG HUNTING				
Fishing region	**Fishing boat**	**Total number of fishermen**	**Number of fishermen interviewed**	**Number of fishermen with catch (hunters)**	**Number of fishermen with catch (hunters)**	**Number of animals taken annually**	**Mean annual catch per hunter**	**95% CI (lower limit)**	**95% CI (upper limit)**	**Number of fishermen with catch (hunters)**	**Number of animals taken annually**	**Mean annual catch per hunter**	**95% CI (lower limit)**	**95% CI (upper limit)**
Eastern	**Inboard**	1658	129	0										
	Non-powered	1276	9	9	0					9	13			
	Outboard-engine	2353	64	19	11	27				15	43			
	Pump-engine		72	39	14	87				36	114			
	All boats	5287	274	67	25	114	4.6	2.61	6.52	60	170	2.8	1.96	3.70
North-eastern	**Inboard**	3084	162	0										
	Non-powered	2374	30	16	0					16	18			
	Outboard-engine	4375	152	52	13	40				49	196			
	Pump-engine		187	81	19	148				77	205			
	All boats	9833	531	149	3	188	5.9	4.10	7.62	142	419	3.0	2.41	3.49
Western	**Inboard**	1795	126	0										
	Non-powered	1382	24	7	0					7	8			
	Outboard-engine	2548	109	44	1	1				44	112			
	Pump-engine		122	27	5	23				27	86			
	All boats	5725	381	78	6	24	4.0	2.44	5.63	78	206	2.6	2.03	3.27
All regions	**Inboard**		417	0										
	Non-powered	6537	63	32	0					32	40	1.3	0.75	1.76
	Outboard-engine	5032	325	115	25	68	2.7	1.70	3.75	108	351	3.3	2.69	3.82
	Pump-engine	9276	381	147	38	258	6.8	5.14	8.43	140	404	2.9	2.31	3.46
Grand total		**20845**	**1186**	**294**	**63**	**326**	**5.2**	**4.01**	**6.34**	**280**	**796**	**2.8**	**2.47**	**3.21**

Table 3. Bootstrap estimated total number of marine mammals killed annually by fishermen in Sabah, with 95% confidence intervals[a,b].

Fishing region	Fishing boat	Total number of fishermen[a]	Number of fishermen interviewed	Estimated total annual dolphin catch	95% CI (lower limit)	95% CI (upper limit)	Estimated total annual dugong catch	95% CI (lower limit)	95% CI (upper limit)
Eastern	**Outboard**	2353	136	1932	1031	3127	2800	1854	3941
	Non-powered	1276	9	0	0	0	865	723	957
	Total	3629	145	1931	1042	3097	3676	2686	4835
North-eastern	**Outboard**	4375	339	2384	1444	3553	5246	4126	6501
	Non-powered	2374	30	0	0	0	843	494	1242
	Total	6749	369	2390	1458	3549	6096	4902	7367
Western	**Outboard**	2548	231	265	64	515	2212	1608	2920
	Non-powered	1382	24	0	0	0	256	106	428
	Total	3930	255	258	64	514	2463	1824	3200
All regions	**Outboard**	9276	706	4636	3246	6239	10305	8673	12056
	Non-powered	5032	63	0	0	0	1965	1560	2407
Overall		**14308**	**769**	**4626**	**3267**	**6185**	**12279**	**10554**	**14103**

[a] Excluding respondents/fishermen using inboard-powered boat

[b] Western total, Northeastern total, Eastern total, all region outboard and all region non-powered are derived from separate runs of the bootstrap procedure and the figure will therefore not necessarily be exactly equal to the sum of figures from runs using data from single regions or boat-types.

Table 4. Results from binomial GLM for variation in the incidence of hunting between different categories of respondents. The table lists all explanatory variables with significant effects in the final models.

Response variable	Explanatory variable	Coefficient (and St Err)	Z-value	P-value
Hunt marine mammals	Fishing gear – traditional	-0.670 (0.165)	-4.223	**0.0000**
	Boat – outboard-engine	-0.995 (0.270)	-3.356	**0.0008**
	Boat – pump-engine	-0.863 (0.290)	-2.981	**0.0029**
Hunt dolphins	Region – Northeastern	1.371 (0.455)	3.022	**0.0025**
	Region – Eastern	2.125 (0.470)	4.528	**0.0000**
Hunt dugongs	Fishing gear – traditional	-0.761 (0.168)	-4.521	**0.0000**
	Boat – outboard-engine	-1.134 (0.299)	-3.793	**0.0001**
	Boat – pump-engine	-0.981 (0.292)	-3.363	**0.0008**

Table 5. Results from quasi-Poisson GLM of variation in numbers of marine mammals reported to be killed. The table lists all explanatory variables in the final models. Significant terms are indicated in bold face.

Response variable	Explanatory variable	Coefficient (and St Err)	Z-value	P-value
Number of dolphins reported killed	Year 1999	1.035 (0.571)	1.811	0.0705
	Year 2000	-0.448 (0.743)	-0.603	0.5465
	Year 2001	0.741 (0.827)	0.896	0.3707
	Year 2002	-0.123 (0.915)	-0.134	0.8933
	Year 2003	0.601 (0.660)	0.911	0.3629
	Year 2004	0.190 (0.780)	0.243	0.8082
	Region – Northeastern	1.605 (0.569)	2.822	**0.0049**
	Region – Eastern	2.089 (0.605)	3.451	**0.0006**
	Boat – pump-engine	1.222 (0.332)	3.686	**0.0002**
Number of dugongs reported killed	No effect			

Species sighted/hunted

Almost all of the respondents (1103, 93%) could readily distinguish between a dugong and a dolphin, and 1032 (87%) respondents regarded the animals as large predatory "fishes". All 63 dolphin hunters interviewed were Bajau and full-time fishermen. Based from illustrations in the field guides and poster, and the hunters' descriptions of the animals, 60 (95%) of the total hunters identified spinner dolphins, 44 (70%) identified bottlenose dolphins, and 18 (29%) identified spotted dolphins as their catches. In all regions, respondents also reported encountering larger cetaceans (e.g., pilot whale, sperm whale, killer whales, and baleen whales) sometimes at sea but had never attempted to hunt the animals, which they claimed were much bigger than their boats. The Irrawaddy and Indo-Pacific humpback dolphins, which were reported common in bays and estuaries, were also not hunted. They believed bad luck would come to those who disturb or harm the species. Other animals reported hunted were large rays (61%), turtles (48%) and whale sharks (13%).

Marine mammal hunting was reported from all 16 fishing districts, except in Tuaran and Beaufort, where dugong was the only species reported hunted. Dugongs were hunted at night, especially during the new moon periods, when the animals came to feed on seagrass in shallow waters close to shore. To hunt dolphins, hunters went out early in the morning when the sea was said to be relatively calm and dolphins were usually found close to coastal islands or reefs to feed. Six hunters (10%) reported to have hunted dolphins at night during their fishing trips.

Hunters used small non-powered, 10 – 25HP outboard- or water pump-engine boats (or commonly known as 'pump boats') for hunting marine mammals (Figure 2). In the Eastern and Northeastern regions, a pump boat is often fitted with a sail and outriggers, which allows it to be used efficiently and enables it to withstand rough sea condition. Overall, half of the hunters used pump boats and hunters using non-powered boats only reported dugong hunting (Table 2).

Four types of gears were reportedly used to hunt marine mammals; harpoon or spear, a combination of harpoon/spear and dynamite, dynamite, and fishing net. Specially made harpoons were used by 38 (60%) dolphin and 181 (65%) dugong hunters (Figure 3). These harpoons were locally known as "bujak", "tempuling" or "sangkir", depending on localities (Figure 4). Twenty (32%) dolphin and 61 (22%) dugong hunters interviewed reported using a combination of harpoon and dynamite, and 5 (8%) dolphin and 27 (10%) dugong hunters used only dynamite to catch the animals (Figure 3). The other 11 (4%) hunters reported using fishing nets to catch dugongs.

On 29 April 1999, three Bajau Pelauh from Semporna were caught by the Police in possession of 12 dead spinner dolphins. Although neither a harpoon nor dynamite were found in their boat, they later confessed that the dolphins were hunted using the combination of the gears. Examination of the carcasses found several wounds made by sharp object and swollen areas, particularly on the dorsal side.

All marine mammal hunters interviewed claimed that the meat and parts of caught animals were consumed by their family members. Two hundreds and fourteen (73%) hunters shared their catches among neighbours. One hundred (34%) hunters said they traded the meat/animals and 86 (29%) hunters reported using the meat/parts as shark baits.

Figure 2. Water pump-engine boats, or commonly known as "pump-boats", fitted with sails and outriggers, used for fishing and/or hunting marine mammals offshore of Semporna, Sabah.

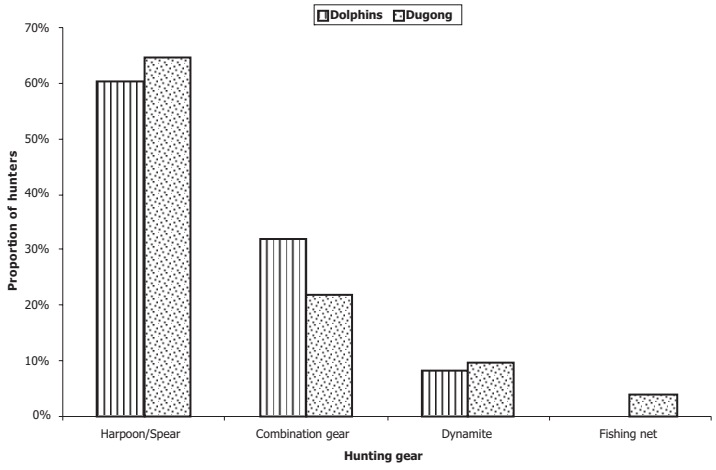

Figure 3. The proportion of marine mammal hunters using different type of hunting gears.

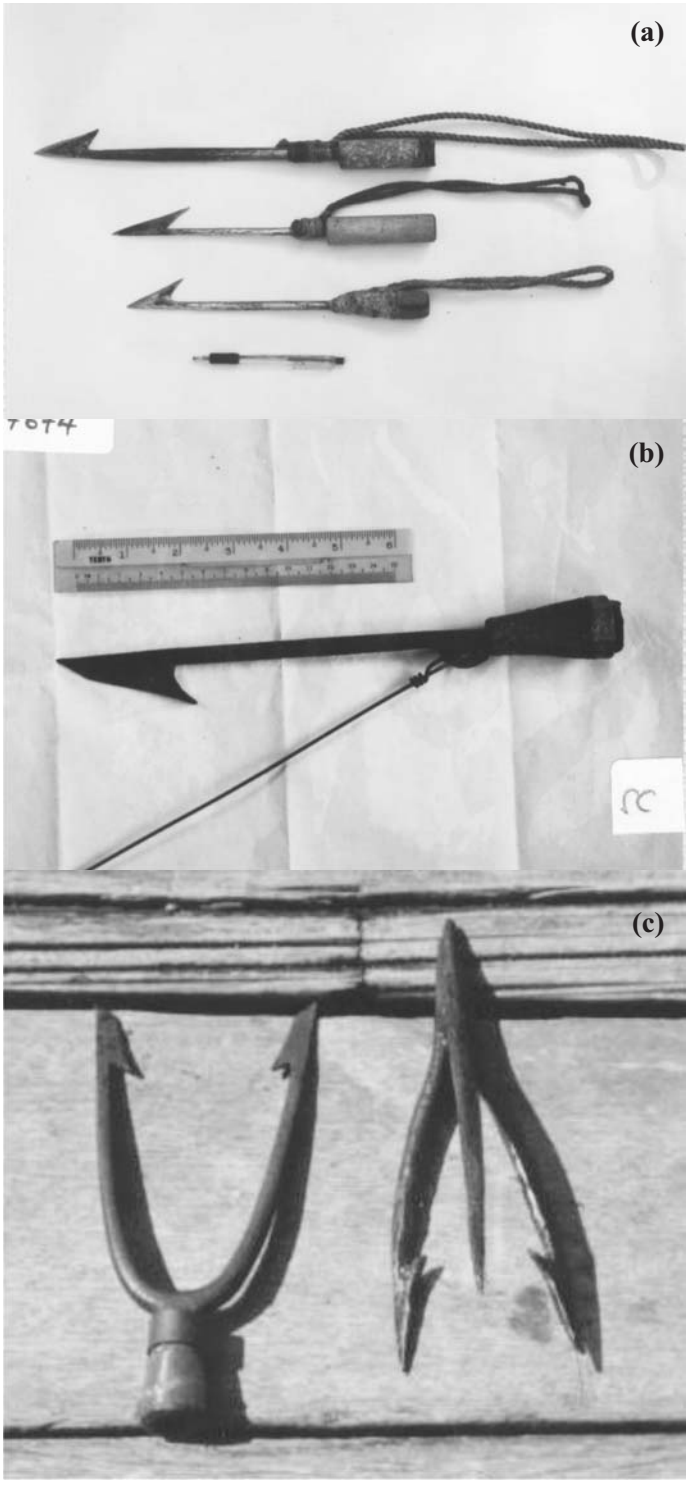

Figure 4. Harpoons, or locally known as (a) "bujak", (b) "tempuling" or (c) "sangkir", used to kill large fishes, turtles or marine mammals in Sabah.

Present utilisation

Two hundreds and sixty-two (89%) hunters interviewed said that they or their fathers/grandfathers had stopped hunting marine mammals mostly in the 1980's and only 32 (11%) said they still hunt dolphins or dugong, at least occasionally, or opportunistically during fishing trips. All hunters who said they presently hunt marine mammals were Bajau and 24 (75%) of them hunt dugongs, two (6%) hunt dolphins and six (19%) hunters take both species. Based on the percentage of present hunters and the estimated total number of marine mammals caught annually by fishermen in each category in the past (Table 3), there is presently an overall estimate of 587 dolphins (95% CI = 415 – 785) and 1,316 dugongs (95% CI = 1,131 – 1,511) caught per year (Table 6).

Table 3. Bootstrap estimated total number of marine mammals killed annually by fishermen in Sabah, with 95% confidence intervals[a,b].

Fishing region	Fishing boat	Total number of fishermen[a]	Number of fishermen interviewed	Number of fishermen still hunting dolphin	Estimated total annual dolphin catch	95% CI (lower limit)	95% CI (upper limit)	Number of fishermen still hunting dugong	Estimated total annual dugong catch	95% CI (lower limit)	95% CI (upper limit)
Eastern	**Total**	3629	145	5	52	13	103	12	493	365	640
North-eastern	**Total**	6749	369	3	224	137	333	14	601	483	726
Western	**Total**	3930	255	0	0	0	0	4	189	138	248
All regions	**Outboard**	9276	706	8	589	412	792	21	873	734	1021
	Non-powered	5032	63	0	0	0	0	9	553	439	677
Overall		**14308**	**769**	**8**	**587**	**415**	**785**	**30**	**1316**	**1131**	**1511**

[a] Excluding respondents/fishermen using inboard-powered boat

Binomial GLM confirmed the existence of significant effects of interview year, region, fishing gear-type and boat-type on present marine mammal hunting (Table 7). A higher proportion of hunters interviewed in 1997 said they still hunt marine mammals, as compared to hunters interviewed in 1999. A higher proportion of hunters in the Eastern region admitted to presently hunt marine mammals than hunters in the Western region. A higher proportion of hunters using gillnet for fishing said they still hunt marine mammals, as compared to hunters using traditional fishing gear. A higher proportion of hunters with non-powered boats said they still hunt the animals, as compared to hunters using boats with outboard- and pump-engines.

Nine hundreds and sixty-one (81%) respondents and 23 (72%) hunters interviewed were aware of the government regulation on fisheries (Fisheries Act 1985) imposed in the 1980's, which include bans on fishing, catching, or selling of any marine mammal species found in Malaysia waters. Binomial GLM confirmed the existence of significant effect of region on respondents' awareness of these regulations and bans. A lower proportion of respondents in the Eastern and Northeastern regions were aware of fisheries regulations and bans, as compared to respondents in the Western region. There were no effects of interview year, ethnic origin, fishing gear-type and boat-type.

Nevertheless, 239 (91%) hunters who had stopped hunting did not give these regulations as their main reason but cited the low availability of marine mammals. Most of the respondents (995, 84%) admitted that the number of dolphins and dugongs has dropped significantly in the past few decades. The dugong, in particular, was reported rarely seen in areas where it was once common, while the dolphins were reported often avoiding fishing boats.

Table 7. Results from binomial GLM for variation in the incidence of hunting between different categories of hunters/respondents. The table lists all explanatory variables in the final models. Significant terms are indicated in bold face.

Response variable	Explanatory variable	Coefficient (and St Err)	Z-value	P-value
Present hunting (hunters)	Year 1999	-1.965 (0.777)	-2.529	**0.0114**
	Year 2000	-0.921 (0.840)	-1.097	0.2725
	Year 2001	-1.440 (1.092)	-1.318	0.1874
	Year 2002	-1.088 (0.904)	-1.203	0.2288
	Year 2003	-0.884 (0.817)	-1.082	0.2792
	Year 2004	-0.681 (0.847)	-0.805	0.4211
	Region – Northeastern	0.490 (0.743)	0.660	0.5094
	Region – Eastern	1.732 (0.840)	2.064	**0.0390**
	Fishing gear – traditional	-1.091 (0.556)	-1.963	**0.0496**
	Boat – outboard-engine	-1.421 (0.651)	-2.184	**0.0290**
	Boat – pump-engine	-2.846 (0.714)	-3.985	**0.0000**
Aware of fisheries regulations/bans (respondents)	Region – Northeastern	-0.745 (0.235)	-3.169	**0.0015**
	Region – Eastern	-1.213 (0.268)	-4.536	**0.0000**

DISCUSSION

This study was based on an interview survey. Lien *et al.* (1994) suggested that interview surveys are not necessarily a reliable source of quantitative data on marine mammal catches, especially if fishermen wish to conceal the occurrence of such mortality. However, this survey method offers a means of obtaining a minimum estimate for numbers of animals killed (López *et al.,* 2003). Interviews can also provide a variety of other useful data, e.g. about attitudes of fishermen towards marine mammals.

During interview sessions, at least an officer from relevant local authorities was present. However, respondents mostly did not conceal the occurrence of marine mammal hunting in the past or at present and readily spoke about how hunting was carried out and what they do with a caught animal. This is probably because it was the first time they were asked such questions and they had not experienced punishment from the authorities for catching dolphins and/or dugongs, though the majority of them knew that hunting marine mammals is illegal. Dolar *et al.* (1994; 1997), Marsh *et al.* (1995) and Persoon *et al.*, (1996) who conducted marine mammal-fisheries interaction interviews in the Philippines, Palau and Aru Islands, Indonesia, respectively, also reported the willingness of respondents in relaying information regarding marine mammal hunting and utilisation in their area. Nevertheless, results of this study need to be viewed cautiously and may be regarded as providing, at best, a rough guide to the scale of marine mammal hunting in East Malaysia.

Magnitude of the Marine Mammal Catches

The present study suggests that substantial numbers of dolphins and dugongs have been and probably continue to be caught for human consumption in Sabah waters. Hunting in the past was a deliberate activity; however, currently there are very few hunters left and hunting is more an opportunistic activity during fishing trips. This new evidence supports the previous suggestions made on the nature and magnitude of dolphin and dugong hunting in the Southeast Asian region. Perrin *et al.* (1996) stated that directed catches for cetaceans in the Southeast Asia are restricted to what appear to be low levels of sporadic take of inshore species in several areas. However, they suggested that the extent is likely underestimated. Recently, Perrin *et al.* (2005) added that significant dugong catches, either intentionally or opportunistically, for subsistence purposes are likely to continue in its range in the region.

The results indicate that the magnitude of dugong catches in the past was similar throughout Sabah, but significantly greater for dolphins in the Eastern and Northeastern regions than in the Western region. The present level of marine mammal hunting in the two regions is still a serious cause for concern since there are significantly higher proportions of respondents that were not aware of government bans on catching marine mammals, as compared to respondents in the Western region. The Eastern and Northeastern regions are apparently larger, less urbanised and a higher proportion of their populations do not receive formal education, as compared to the Western region (TRPDS, 1998). This might explain why people there are less aware of laws and matters pertaining to environmental conservation.

Fishermen using outboard- or pump-engines boats hunted both marine mammal species, whereas those using non-powered boats were able to hunt only dugongs. Pump boats were preferred, being used by more than half of the fishermen, and the number of dolphins reported to be killed is significantly higher for fishermen using pump boats than boats with outboard-engines. Since pump boats are used by the majority of hunters in the Eastern and Northeastern regions, this could also explain the higher estimated catch of dolphins in these regions, as compared to the Western region. Small to medium size pump boats are also used by cetacean hunters in the Philippines (Dolar *et al.*, 1994; 1997) and hunters in Lembata and Solor Islands, Indonesia, used nine- to fifteen-man sail or outboard-engine boats to hunt whales, dolphins and other large marine species (Barnes, 1991; 2005). Outboard-engine boats are also used by dugong hunters in the Torres Strait and Palau, while dugout canoes are used to hunt dolphins and dugongs in the Solomon Islands (Takekawa, 2000) and Aru Islands, Indonesia (Persoon *et al.*, 1996), respectively.

The results also indicate that harpoon/spear is the main gear used to hunt marine mammals. Although there is some difference in shape and operating method, the finding is consistent with other reports on marine mammal hunting in the region (Barnes, 1991; 2005; Dolar *et al.*, 1994; Persoon *et al.*, 1996). It is not illegal to carry a harpoon at sea, thus many fishermen keep it in their boats and use it usually to kill sharks or turtles that are caught in their fishing gears. However, when an opportunity arises, such as a dugong being spotted close by or incidentally caught in fishing net, they are most likely to use the harpoon to hunt/kill the animal. The use of dynamite to catch fish, is illegal, but sometimes it is also used to kill marine mammals. This home-made bomb, which uses fertiliser as it main component, has devastated many reef areas in Sabah and other parts of the Southeast Asia and continues to be used, with a lack of commitment by the authorities to control its usage (Perrin *et al.*, 2005; Oakley *et al.*, 2000).

In addition, the legal system often shows leniency in relation to these kinds of crimes. According to Sabah Wildlife Conservation Enactment (1997), any person who kills a protected species (e.g. a cetacean) shall be liable on conviction to a maximum fine not exceeding RM50,000 (US$12,500) or to imprisonment for five years or to both. In the case of the three Bajau Pelauh who were caught by the Semporna Police for hunting 12 spinner dolphins, they were given only six months jail term. In other cases when marine mammals or the meat/parts were confiscated by officers from relevant local authorities, often the culprits were released with warnings. Thus, hunting and trading of marine mammals in the rural areas probably continues unabated due to a lack of commitment in enforcing conservation laws and the low risk of getting a significant punishment. Similar situations are also reported to occur in the Philippines, Palau, the Torres Strait and Aru Tenggara Marine Reserve (Dolar *et al.*, 1994; 1997; Marsh *et al.*, 1995; 1997; Persoon *et al.*, 1996).

Sustainability of the Marine Mammal Fishery

There is no estimate of marine mammal populations in Malaysian waters. However, the cetacean population is assumed to be small in numbers, as suggested for the populations in other countries in the Southeast Asian region (Perrin *et al.*, 1996; 2005). While the status of the dugong population is not known for any country in the region, numbers are believed to have declined throughout the region with the possible exception of Australian waters (Perrin *et al.*, 2005).

With the exception of the Eastern region, the annual total number of dugongs reported taken by hunters and the estimated number of dugongs taken by fishermen in other regions and boat-type categories are more than double than the totals for dolphins. This shows that dugong is much preferred and probably more common in the coastal waters than dolphins. Lah-Anyi and Jaaman (2002) found that dugongs were more commonly stranded than dolphins between 1996 and 2001. The spinner, bottlenose, and spotted dolphins that were identified as catches were reported to be abundant in the open waters (Beasley, 1998; Jaaman *et al.*, 2001; Jaaman, 2004). Nevertheless, there is a possibility that respondents have mistakenly identified the species with other members of the Delphinidae family (e.g. striped, common, or Fraser's dolphins), which also could be abundant in the open waters. Only dugong and spinner dolphin carcasses were recovered from a number of hunters and fish traders during the survey period.

Respondents and hunters admitted that the number of dolphins and dugongs has dropped significantly in the past few decades. Though they are aware of the basic facts with regard to the physical requirements of the dugong, most of them refused to accept the possibility of extinction due to the disturbance and hunting pressure. They tend to believe that the animal is capable of hiding in safer places, even though they know that dugong live off seagrass, which they said is less common in the coastal waters now. The same thought has also been recorded from dugong hunters in Aru Islands, Indonesia, about the animal's population in their area (Persoon *et al.*, 1996).

Although marine mammal hunting in the past was a deliberate activity, hunters were probably opportunists and would kill other large species, such as the manta ray, turtle or whale shark, when the opportunity arose. As the chances of catching marine mammals are very small nowadays, only very few hunters still go out to hunt dolphins (8 hunters) or dugong (30 hunters). Marsh *et al.* (1997) reported that in the Torres Strait, between Australia and Papua New Guinea, dugongs and green turtles are hunted together and hunting would be expected to cease only when the combined density of the two species is so low that hunting is not worthwhile. Besides taking a large numbers of fish, many coastal villagers in Lembata and Solor Islands, Indonesia occasionally took dugong (Barnes, 2005). The situation is similar in Sabah, where opportunistic hunting of several large marine species continues, especially during fishing trips, depending on availability. In addition, many fishermen bring harpoon, or worst still dynamite, during fishing, thus there is a real danger of dolphin and dugong populations being seriously affected by opportunistic hunting unless the use or harpoon and dynamite is stopped.

The annual total number of dolphins and dugongs reported to be taken by the respondents and the estimated number of animals taken by fishermen in each region and boat-type categories in the past is considered very unsustainable. At the second meeting of the Agreement on the Conservation of Small Cetaceans of the Baltic and North Seas (ASCOBANS), in 1997, it was agreed that, in general, an anthropogenic removal of more than 2% of the best available cetacean population estimate was an "unacceptable interaction" (ASCOBANS, 1997). Based on a dedicated sighting survey in the Southern Sulu Sea of the Philippines, there is an estimated 3,979 (CV=0.59) spinner dolphins, 3,455 (CV=0.32) pantropical spotted dolphins, and 415 (CV=0.96) bottlenose dolphins in the area (Dolar *et al.*, 1997). Assuming that fishermen in the Northeastern region are catching dolphins from the same population and the 2% of anthropogenic removal is exclusively from hunting activities, the maximum sustainable catch will be 80 spinner dolphins, 69 pantropical spotted dolphins, and 8 bottlenose dolphins, or a total of 157 dolphins taken annually. The annual total number of dolphins reported taken by the respondents in the Northeastern region (Table 2) and the estimated number of dolphins taken by fishermen in any of the regions (Table 3) substantially exceeds this figure. Furthermore, this dolphin population is also subject to by-catches in fisheries (S. A. Jaaman, unpublished data), and directed and incidental catches in the neighbouring Philippines waters (Dolar, 1994; Dolar *et al.*, 1994; 1997).

In the case of dugong hunting, the population simulations of Marsh (1995; 1999) suggested that the sustainable level of exploitation may be as low as 2% of females annually. If five females are taken in any of the regions each year, at least 250 female dugongs would be needed in the waters of each region for the population to be maintained, which is considered to be extremely unlikely. A similar suggestion has been made concerning the maintenance of dugong populations in Palau waters (Marsh *et al.*, 1995) and in some other areas within the species' range (Marsh *et al.*, 2002). The present utilisation level of an estimated 224 dolphins (95% CI = 137, 333) and 601 dugongs (95% CI = 483, 726) per year in the Northeastern region is also unsustainably high.

The estimated number of dolphins or dugongs taken annually for consumption by fishermen in each region and boat-type categories is extremely high given the large quantities of fishes landed (175,122 metric tons) from the coastal waters (DFS, 2004). These directed catch estimates suggest some or all of the following: (1) the numbers of dolphins or dugongs reported taken by the respondents are too high; and (2) the respondents are not representative in each category.

Conservation recommendations

The introduction of Fisheries Act 1985 appears to have had little effect on directed catches of marine mammals in Sabah, East Malaysia. Several factors are evident, but the main ones are poor enforcement and the low risk of getting a significance punishment for catching the protected animals. There is apparently a shortage of manpower and proper equipment, limited funding and little initiative to conduct marine environmental conservation and management programs in Sabah. Although hunting nowadays is an opportunistic activity, it could still seriously affect dolphin and dugong populations, which are assumed to be low in numbers.

Therefore, it is suggested that the management and enforcement authorities and the community leaders should act promptly to establish a collaborative and dedicated monitoring program to identify significant directed catches of marine mammals in Sabah. This program should focus on minimising the threats through education with the backup of heavy penalties for contravention of regulations. However, this has to be done cautiously due to the fact that in some countries, enforcement of laws prohibiting direct takes or landing of incidental catches of marine mammals has increased the difficulty in obtaining information on such takes (Perrin *et al*., 1996; 2005). Furthermore, enforcing law within a large area is often difficult and costly, thus it is essential to educate coastal communities towards compliance with fishing regulations and conserving their environment. This is an alternative to enforcement and would encourage their involvement in species monitoring.

Fishing communities should also be given alternative livelihoods, such as in mariculture or ecotourism sectors, if they have to reduce/stop fishing. Furthermore, the economic and social values of marine mammals among certain coastal communities that hunt and utilise the animals need to be investigated further. These communities should also have a significant role in any management, conservation and/or utilisation of natural resources in their areas.

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DETECTING OUTLIERS BY USING TRIMMING CRITERION BASED ON ROBUST SCALE ESTIMATORS WITH SAS PROCEDURE

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ABSTRACT. *Non-normal data and heteroscedasticity are two common problems encountered when dealing with testing for location measures. Non-normality exists either from the shape of the distributions or by the presence of outliers. Outliers occur when there exist data values that are very different from the majority of cases in the data set. Outliers are important because they can influence the results of the data analysis. This paper demonstrated the detection of outliers by using robust scale estimators such as MAD_n, T_n and LMS_n as trimming criteria. These criteria will trim extreme values without prior determination of trimming percentage. Sample data was used in this study to illustrate how extreme values are removed by these trimming criteria. We will present how these were done in a SAS program.*

KEYWORDS. Outliers, trimming, robust scale estimator.

INTRODUCTION

Analysis of variance (ANOVA) is the most commonly used statistical method for locating treatment effects in one-way independent groups design. However, ANOVA can be adversely affected by two general problems. The problems are non-normal distribution and heteroscedasticity. When these two problems arise simultaneously, rates of Type I error are usually inflated resulting in spurious rejection of null hypotheses, and reduction in the power of the test statistics.

Departures from normality originate from two problems, i.e. skewness and outliers. These problems may be remedied by using transformation such as exponential, logarithm and others but sometimes, even after the transformation, problems with non-normal data still occur. Simple transformations of the data such as by taking logarithm can reduce skewness but outliers may not be eliminated. According to Wilcox and Keselman (2003), simple transformations can alter skewed distributions to make them more symmetrical, but they still do not deal directly with outliers. They also stated that to deal directly with outliers and eliminate deleterious effects, one has to do trimming.

Trimming

Trimming is a method to eliminate outliers or extreme observations from each tail of a distribution. This method can be highly beneficial in terms of efficiency and in achieving high power. According to Wilcox (1998) the effectiveness of trimming depends on the criteria we adopt and the goals that we hope to achieve.

Nevertheless, when the sample size, n is small, the optimal amount of trimming is yet to be determined. Rocke, Downs and Rocke (1982) in their paper concluded that the best result were obtained with 20% – 25% symmetric trimming, Othman, Keselman, Padmanabahan, Wilcox and Fradette (2004) reported that one can achieve a slightly better Type I error control with a 15% symmetric trimming rather than a 20% symmetric trimming. Keselman, Othman, Wilcox and Fradette (2004) demonstrated that a good control of Type I error can be achieved with only modest amounts of trimming, namely 15% or 10% from each tail of the distribution. Despite these observations, to empirically determine how much trimming that is needed, is difficult and not always obvious.

Another approach is not to trim a fixed amount of the data but only the outliers. Othman, Keselman, Wilcox, Fradette and Padmanabhan (2002) in their study reported from the theoretical considerations that when data are said to be skewed to the right, then in order to achieve robustness to non-normality and greater sensitivity to detect effects, one should trim data just from the upper tail of the data distribution. There are various ways to check for outliers and the choice of method can result in a significant difference.

METHODS

This paper focuses on several trimming criteria using robust scale estimators such as MAD_n, T_n and LMS_n (Rousseeuw & Croux, 1993). By using these methods, the number of outliers to be discarded will be determined before further analysis can be performed.

Trimming criterion

We calculated the number of outliers by using:

i_1 = number of observations X_{ij} such that $\left(X_{ij} - \widehat{M}_j\right) <$ -K (scale estimator),

i_2 = number of observations X_{ij} such that $\left(X_{ij} - \widehat{M}_j\right) > K$ (scale estimator),

$\widehat{M}_j$ = median of group j and the scale estimator can be MAD_n, T_n and LMS_n.

The constant $K = 2.24$ is motivated in part by the goal of having reasonably small standard error when sampling from a normal distribution (Othman, *et al.,* 2004).

Robust scale estimators

Scale measure is a quantity that explains the dispersion of a distribution. The value of a breakdown point is a main factor to be taken into account for a fine scale estimator (Wilcox, 1997). Rousseeuw and Croux (1993) have introduced several scale estimators by considering their high breakdown point.

MAD_n, T_n and LMS_n are three robust scale estimators adopted in this study. These estimators have 0.5 breakdown value and also exhibit bounded influence functions. In addition to the good characteristics of these robust scale estimators, they are simple and easy to compute.

***MAD*$_n$**

MAD_n is the median absolute deviation about the median. It has the best possible breakdown value and its influence function is bounded with the sharpest possible bound among all scale estimators (Rousseeuw & Croux, 1993). However, there are also some drawbacks about this scale estimator. The efficiency of MAD_n is very low with only 37% at Gaussian distribution. MAD_n takes a symmetric view on dispersion and also does not seem to be a natural approach for asymmetric distributions.

Given $x_{(1)j} \le x_{(2)j} \le \ldots \le x_{(n_j)j}$ are the ordered sample of group j with size n_j, this robust scale estimator is given by

$$MAD_{nj} = b \ \text{med}_i \left| x_{(ij)} - \widehat{M}_j \right| \qquad [1]$$

where the constant $b = 1.482$ is needed to make the estimator consistent for the parameter of interest.

***T*$_n$**

Another scale estimator proposed by Rousseeuw and Croux (1993) is T_n, which also has the highest breakdown point like MAD_n. The scale estimator for group j is given as

$$T_n = 1.3800 \frac{1}{h_j} \sum_{k=1}^{h_j} \left\{ \underset{i \neq i'}{med} \left| x_{(i)j} - x_{(i')j} \right| \right\}_{(k)} \qquad [2]$$

where $h_j = \left[\frac{n_j}{2} \right] + 1$ and $[x]$ returns the largest integer less than or equal to x. T_n was proven to have 50% breakdown point and an efficiency of 52%, which is more efficient than MAD_n.

***LMS*$_n$**

LMS_n is also a scale estimator with a 50% breakdown point and is based on the length of the shortest half sample as shown below:

$$LMS_{nj} = c' \min_i \left| x_{(i+h-1)j} - x_{(i)j} \right| \qquad [3]$$

The default value of c' is 0.7413 which achieves consistency at Gaussian distributions (Rousseeuw & Croux, 1993). The estimator [3] first occurred as the scale part of the least median of squares (LMS) regression estimator (Rousseeuw, 1984) in the special case of one-dimensional data.

RESULTS AND CONCLUSION

Table 1 shows the data used in this study. The data were from three different groups with sample sizes of, $n_1 = 15$, $n_2 = 10$ serta $n_3 = 10$. Table 2 illustrates the procedure used in this study in the form of SAS pseudo codes. The codes were run using SAS IML procedure to identify the number of outliers in a group.

Table 1. Sample data with $N = 35$ ($n_1 = 15$, $n_2 = 10$, $n_3 = 10$)

Groups	Data
1	26 34 46 48 42 49 74 61 51 53 26 34 46 48 42
2	51 50 33 28 47 50 48 60 71 42
3	52 64 39 54 58 53 77 56 63 59

The robust scale estimators used in this procedure are MAD_n, T_n and LMS_n. By using these procedures, the number of extreme values that need to be removed can be identified.

Table 2. Pseudo codes of SAS IML procedure for trimming criterion using robust scale estimator

```
DO I = 1 TO NCOL(NX);
                SAMP = NX[I];
                L = M + SAMP;
                TEMP1=Y[F:L];

        **GROUP CRITERIA FOR TRIMMING **;
                GCRIT = 2.24 # (SCALE ESTIMATOR);
        **GETTING GROUP MEDIANS **;
                GMED[,I] = MEDIAN(TEMP1);
        **GETTING Y – GROUP MEDIAN **;
                TEMP2=TEMP1-GMED[,I];
        **SORTING Y AND Y – GROUP MEDIAN**;
                NV1 = TEMP1;
                TEMP1[RANK(NV1),] = NV1;
                NV2 = TEMP2;
                TEMP2[RANK(NV2),] = NV2;

*DETERMINING EXTREME VALUES ON THE LEFT AND RIGHT TAIL OF THE
DISTRIBUTIONS *;
 LEFT = (TEMP2 < -1 # GCRIT[,I]);
 I1VEC[,I] = SUM(LEFT);
 RIGHT = (TEMP2 > GCRIT[,I]);
 I2VEC[,I] = SUM(RIGHT);
```

Table 3 shows the number of outliers detected based on different trimming criterion. As we can see in Table 3, the trimming criterion using MAD_n has the highest number of outliers. For this trimming criterion, two outliers were detected on the left tail for each set of group 1 and group 2 while on the right tail, all the three groups had one outlier each. If we look at the trimming criterion using LMS_n, no outlier were detected on both tails.

Table 3. Number of outliers

Scale estimator	MAD_n		T_n		LMS_n	
Trimming	Left	Right	Left	Right	Left	Right
Group 1	2	1	0	1	0	0
Group 2	2	1	0	0	0	0
Group 3	1	1	1	1	0	0

Trimming is a method to eliminate outliers or extreme observations from each tail of a distribution. Determining the percentage of trimming must be made prior to the testing. In order to make this decision, efficiency is one factor to be considered. In this context, efficiency means achieving relatively small standard error when the trimming method is used. Trimming need to be done cautiously. If the amount of trimming is too small, efficiency can be very poor when sampling is from heavy-tailed distribution, but if the amount is too large, efficiency will be very poor when we consider the sampling from a normal distribution (Keselman, Kowalchuk, Algina, Lix, & Wilcox, 2000).

By using this SAS procedure, the process of detecting outliers is made simple and convenient for researchers and this will also give them an option to decide whether to discard the values or to use them in their research.

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DETERMINATION OF ON-LINE BREAKTHROUGH VOLUME AND PERCENTAGE RECOVERY OF MODIFIED POLYSTYRENE-DIVINYLBENZENE (PS-DVB) AS CHROMATOGRAPHY ADSORBENT

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ABSTRACT. *The breakthrough volume is the most important characteristic parameter to determine the suitability of a sampling device for isolating the analytes of interest. The home-made solid phase extraction-ultraviolet (SPE-UV) detection system has been developed for the determination of breakthrough volumes of adsorbents. The system consists of an HPLC pump that delivers the analyte, a stainless steel precolumn that contains the adsorbent, a selection valve, and a UV detector. This system was used to study the capacity and affinity anatytes trapped in the modified PS-DVB adsorbents synthesized in the laboratory (PS-DVB heptadecyl ketone, PS-DVB chloromethyl, PS-DVB octadecoxy methyl) and commercial PS-DVB and ODS-silica adsorbents using nitrobenzene and 2-chlorophenol as the test compounds. The highest breakthrough volumes among the modified adsorbents were achieved for PS-DVB heptadecyl ketone i.e. 36.75 mL at 4 ppm of nitrobenzene and 4.68 mL at 4 ppm of 2-chlorophenol. PS-DVB heptadecyl ketone which shows very good recovery values i.e 110 ± 2.01% for nitrobenzene and 68 ± 2.18% for 2-chlorophenol. This suggest that PS-DVB heptadecyl ketone has greater capacity and affinity in trapping the analytes.*

KEYWORDS. Breakthrough volume, polystyrene-divinylbenzene, chromatography adsorbent.

INTRODUCTION

The ability of solid surfaces to bind molecules of organic compounds via different affinity mechanisms has been known for many decades. The analytical possibilities offered by this phenomenon were gradually recognized during long-term development of chromatographic techniques first introduced by Tswett at the turn of the twentieth century (Liska, 2000). Since then various hydrophilic functional groups were chemically attached to the benzene rings of porous, crosslinked polystyrene resins as stationary phase for SPE.

In SPE, the analytes to be extracted are partitioned between a solid and a liquid rather than between two immiscible liquids as in LLE and these analytes must have a greater affinity for the solid phase than for the sample matrix (retention or adsorption step) (Puig *et al.,* 2007). The choice of adsorbent is therefore a key point in SPE because it can control parameters such as selectivity, affinity and capacity (Fontanals, 2005).

SPE is usually performed using a SPE tube containing appropriate packing. Modified ODS-silica reversed-phase adsorbent is one of the most common and widely used packing materials for SPE because of its greater capacity compared to other bonded silicas, such as the C_8 and CN types (Tang *et al.,* 2008). The mechanism of retention is based on hydrophobic interactions between the solutes and the stationary phase (Van der Waals forces) (Leon-Gonzalez *et al.,* 2000).

Nevertheless, the main drawback of such adsorbents is their narrow pH stability range. Consequently, when SPE has to be carried out in extremely acidic or basic media, reversed-phase polymeric adsorbents (generally based on PS-DVB) are used (Gülbakan *et al.,* 2008). In addition to their broader pH stability range that increase the flexibility of the method, these kind of adsorbents have a greater surface area per gram and they show relative selectivity for analytes with aromatic rings because of their π-π interactions (Fontanals *et al.,* 2008). Owing to their hydrophobicity, they show a poor surface contact with predominantly aqueous solutions during SPE and cause a low breakthrough volume and adsorbent capacity.

The target of creating new types of chemically bonded resins is to overcome these drawbacks. For an improvement it has been shown that introduction of polar groups into a PS-DVB resin greatly increases the retention of polar organic compounds. Sanagi *et al.,* (2005) modified PS-DVB with alcohol and acetyl functional groups which exhibited excellent hydrophilicity and a reduced dependence on wetting prior to extraction. In general, modified polymer phases have the advantage over bonded silicas that they can be used over the entire pH range.

One important parameter to control in the development of SPE method is the breakthrough volume, which is the sample volume where the analyte starts to elute from the exit of the column. The sample volume indicates the amount of analyte that can be preconcentrated and that is available for detection. The value of breakthrough volume is a function of the chromatographic retention of analyte on the particular sorbent in the SPE column and can only be altered by a change of sorbent (Hennion & Coquart, 1993).

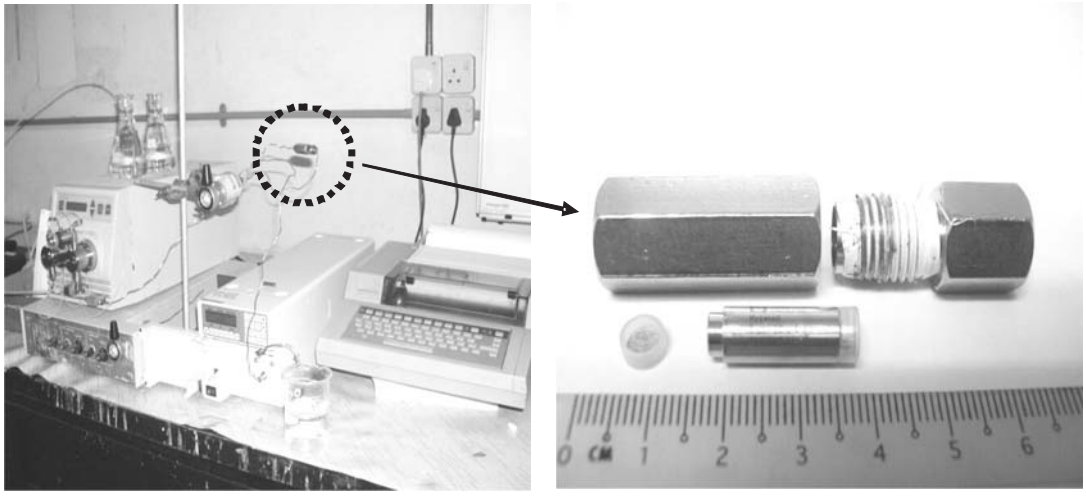
The breakthrough volume can be determined by pumping a dilute solution of the analytes through the stainless steel pre-column connected to a detector through a selection valve (Hyötyläinen, 2007). The breakthrough volume and adsorbent capacity for a given packed bed are valuable information to determine SPE parameters such as adsorbent amounts and bed thickness (Liu *et al.,* 2006). A home-made set-up for breakthrough volume and adsorbent capacity measurement can be utilized for characterization different adsorbents with ease and simplicity, time and cost effective.

The aim of this paper is to determine of breakthrough volume and percentage recovery of two different analytes namely, nitrobenzene and 2-chlorophenol on three different home-made adsorbents PS-DVB heptadecyl ketone, PS-DVB chloromethyl and PS-DVB octadecoxy chloromethyl. The SPE-UV detection system was developed in order to measure the breakthrough volume and percentage recovery of the analytes.

EXPERIMENTAL

Instrumentation

SPE tube in the form of stainless steel precolumn (Supelco, USA) (2.0 mm length and 0.6 mm I.D) was used to pack the sorbents, with an approximate weight of 0.150 g. A Rheodyne six port injection valve was used as switching valve (Cotati, USA) and the stainless steel precolumn being placed in the sample-loop position of the switching valve by using the set-up as shown in Figure 1. The analyte solution was passed through the precolumn by using HPLC pump, JASCO Waters-515 (Tokyo, Japan). Sample solution was introduced to precolumn at 0.1mL/min. UV detector from JASCO (Tokyo, Japan) was use to detect the analyte at 254 nm for nitrobenzene and 280 nm for 2-chlorophenol.



(a) **(b)**

Figure 1. (a) The experimental set up of the SPE-UV detection system. (b) The stainless steel precolumn and the housing.

Breakthrough volume curves were acquired with a JASCO Waters-515 HPLC pump (Tokyo, Japan) and a JASCO Intelligent UV 2075 plus UV/Vis detector (Tokyo, Japan). All measurements were performed at 254 nm for nitrobenzene solution, and 280 nm for 2-chlorophenol solution. Data acquisitions were made using a Hewlett-Packard HP3396A integrator (USA). The percentage recovery of each analyte with different sorbents were carried out by using GC-FID; the analytes eluted from SPE tube were collected and then analysed using a Hewlett Packard Model 6890GC gas chromatography (GC) equipped with a flame ionisation detector (FID) and a data processor (USA).

The gas chromatographic column used was Ultra-1 932530, a non-polar, fused-silica capillary column (30 m length × 250 μm inner diameter × 0.20 μm film thickness) (USA). Helium was used as the carrier gas at a flow rate of 1.1 mL/min at a pressure of 75 kPa. The injector temperature was set at 250°C and the detector temperature at 310°C. The gas chromatography oven was operated under programmed temperature with an initial temperature of 100°C, which was held for 2 minutes and ramped up to 140°C at a rate of 5°C/min (Puig, 2007). Each sample (1 L) was injected into the gas chromatograph by using a 10 μL syringe obtained from Agilent (Little Fall, USA). Three injections were carried out for each sample to obtain a good accuracy.

Solid Phase Extraction (SPE) Precolumn Packing

In this study, a new set-up was developed to obtain the breakthrough volumes and recoveries data. Guard column / precolumn was used in place of traditional SPE polypropylene tubes to pack the sorbent. The original content of the guard column was removed and the column was thoroughly cleaned. For packing purpose the top plastic cap was removed and the sorbent was slowly added (which was assisted by or with the help of syringe barrel that acted as funnel) into the stainless steel precolumn. A small amount of sorbent was added at a time and each time the stainless steel precolumn will be given a gentle tap – to have homogenous/compact packing (Schmidt & Fritz, 1993). This was done until the whole stainless steel precolumn cavity was filled with sorbent (150 mg). The plastic cap that was removed earlier for packing purpose will be fixed to the same position – top end of the stainless steel precolumn. The SPE stainless steel precolumn packing processes is illustrated in Figure 2.

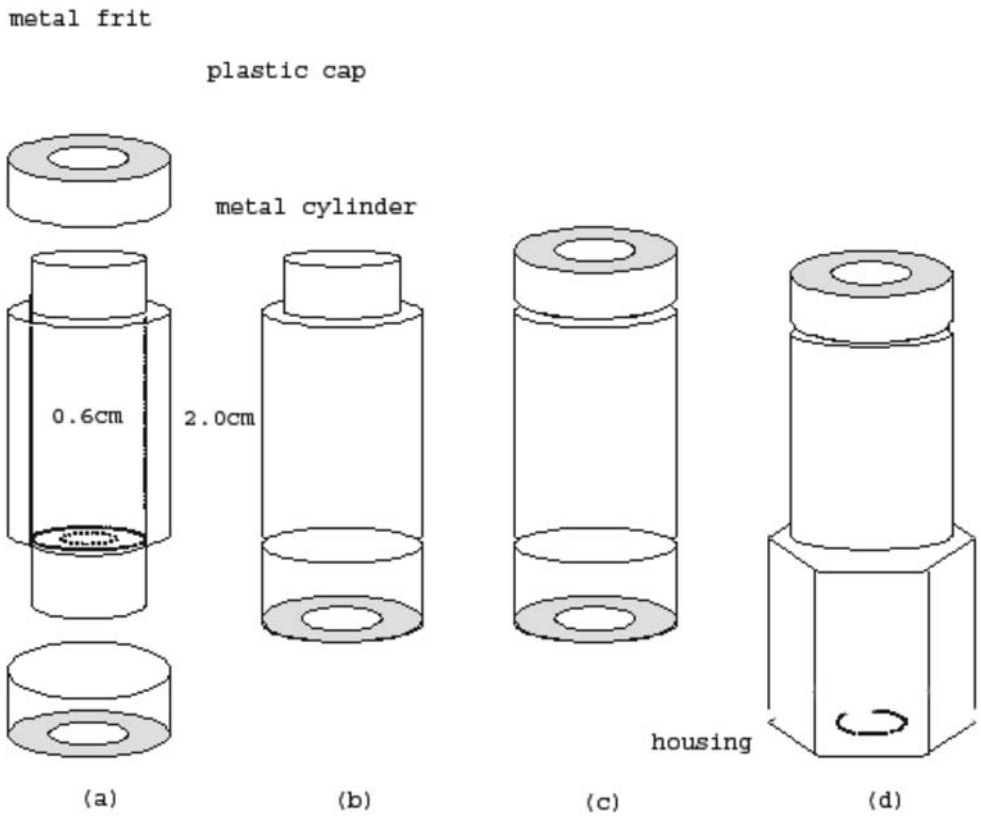


Figure 2. SPE precolumn packing process: (a) Construction of the SPE stainless steel precolumn (b) Cap removed for sorbent filling. (c) SPE stainless steel precolumn fully capped (d) SPE stainless steel precolumn placed inside the housing.

Conditioning of Precolumn

The SPE sorbent was activated / cleaned by pumping 2.0 mL of methanol through the stainless steel precolumn using HPLC pump at a flow rate of 0.5 mL/min. Displacement of methanol was done by using 2.0 mL of deionised water at the same flow rate as above. Upon completing the above steps, the sorbent is ready to receive sample solution. Test solution of 4 ppm 2-chlorophenol and nitrobenzene will be passed through the precolumn at a flow rate of 0.1 mL/min. Selection of the low flow rate is to maintain a low pressure which might interrupt the entrapment of analyte. Once the recording of the breakthrough volume is completed i.e the curve reached 10% of the initial UV absorbance, regeneration of the sorbent will be carried out where the sorbent will be cleaned with methanol and deionised water until it gives zero base line reading on UV detector. The equations used to calculate the breakthrough volumes are as follows:

Breakthrough volumes = Retention time x Flow rate (1)
Retention time = Retention distance / Chart speed (2)

The formula used to calculate the response factor for each of the analyte studied is

$$\text{Response factor, } F_x = \frac{\text{Average peak area of test compound (pA)}}{\text{Concentration of test compound (ppm)}} \quad (3)$$

Breakthrough Volume Measurement Procedure

Test stock solutions of 40,000 ppm were prepared by weighing 1.0 g of each nitrobenzene and 2-chlorophenol separately in two 25mL volumetric flasks and diluted in methanol to 25 mL. The working test solution or sample aqueous solution for each analyte; nitrobenzene and 2-chlorophenol at 4 ppm were prepared by adding 0.2 mL of 40,000 ppm stock solution into two separate 2000 mL volumetric flasks and then diluted to the mark with deionized water. Conditioning of the precolumn was carried out. Solution containing 400 ppm of each test compound was passed through the precolumn at a flow rate of 0.1mL/min for 10 mins. The precolumn was purged with nitrogen gas to remove the water for a duration of 30 mins at 80 psi (Lefebvre, 2007). The precolumn was eluted with 1.0 mL of methanol and the eluate was collected in a centrifuge tube. The internal standard (0.1 mL) was added to the eluate, capped and agitated. The eluate was stored in a refrigerator while waiting for analysis. 1.0 uL aliquot was injected into GC to obtain the chromatogram. The working test solution or sample aqueous solution for each analyte; nitrobenzene and 2-chlorophenol at 400 ppm were prepared by adding 1.0 mL of 40,000 ppm stock solution into two separate 100 mL volumetric flasks and then diluted to the mark with deionized water.

RESULTS AND DISCUSSION

Breakthrough measurements

The retention efficiency of modified PS-DVB and unmodified PS-DVB were determined by measuring the breakthrough volume of the adsorbents. The equations used to calculate the breakthrough volumes are as in equation 1 and 2. The concentration of the analyte used in determining the breakthrough volumes was 4 ppm. Table 1 shows the breakthrough volume of ODS-silica, unmodified and modified PS-DVB sorbents using nitrobenzene and 2-chlorophenol as analytes.

Table 1. Breakthrough volumes for ODS-silica, unmodified PS-DVB and modified PS-DVB sorbents using nitrobenzene and 2-chlorophenol as analytes.

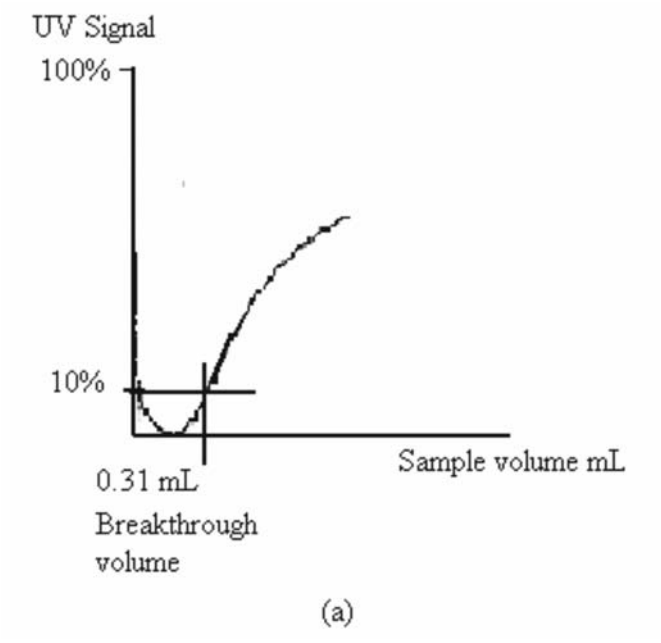
Sorbent	Nitrobenzene 4 ppm		2-Chlorophenol 4 ppm	
	Average breakthrough volume (mL)	RSD (%)	Average breakthrough volume (mL)	RSD (%)
ODS-silica	42.43	1.31	20.99	8.56
PS-DVB commercial	0.31	8.89	0.10	15.80
PS-DVB heptadecyl ketone	36.75	2.69	4.68	21.52
PS-DVB chloromethyl	0.31	5.10	0.16	7.65
PS-DVB octadecoxy methyl	0.12	17.68	0.10	15.81

From Table 1, it is apparent that, the PS-DVB heptadecyl ketone has the highest breakthrough volumes for both analytes compared to those for commercial PS-DVB and modified sorbents used in this study. PS-DVB heptadecyl ketone having the highest capacity in trapping the analytes i.e 0.15 mg of nitrobenzene and 0.02 mg of 2-chlorophenol per 150 mg of sorbent. PS-DVB octadecoxy methyl sorbent capacity was lowest compared to other modified sorbents (Table 2). This may be due to the presence of the polar carbonyl groups on PS-DVB heptadecyl ketone surface, which improved the efficiency of the adsorbent by increasing the ability to undergo polar interactions with the polar analytes. In addition, the presence of polar groups also caused the sorbent to be wetted easily and have an intimate contact with aqueous solution, therefore the analyte can easily be extracted from the solution. Another reason could be that better interactions occurred between solutes (polar and non-polar) and PS-DVB heptadecyl ketone sorbent owing to higher surface area of the sorbent (the home-made PS-DVB have been synthesized using 8% of crosslinker whereas the commercial PS-DVB only 4%).

Table 2. Sorbent capacity of ODS-silica, unmodified PS-DVB and modified PS-DVB sorbents using nitrobenzene and 2-chlorophenol as analytes.

Sorbent (150mg)	Nitrobenzene 4 ppm		2-Chlorophenol 4 ppm	
	Sorbent capacity (mg)	RSD (%)	Sorbent capacity (mg)	RSD (%)
ODS-silica	0.17	1.31	0.08	8.56
PS-DVB commercial	$1.24x10^{-3}$	8.89	$0.4x10^{-3}$	15.80
PS-DVB heptadecyl ketone	0.15	2.69	0.02	21.52
PS-DVB Chloromethyl	$1.24x10^{-3}$	5.10	$0.64x10^{-3}$	7.65
PS-DVB Octadecoxy methyl	$0.48x10^{-3}$	17.68	$0.40x10^{-3}$	15.81

The breakthrough volume for nitrobenzene and 2-chlorophenol were 0.30 mL and 0.10 mL respectively. Figure 3 shows the breakthrough curve of nitrobenzene and 2-chlorophenol using commercial PS-DVB. Based on the results obtained, the analytes were poorly retained on the said sorbent. The nature of analytes plays an important role in retention mechanisms on the PS-DVB which involves the π-π interactions between the analyte and sorbent. PS-DVB possesses exceptionally strong π-electron donating-accepting ability, which causes retention of compounds that contain aromatic π-systems or functional groups with lone electron pairs such as carbonyl and nitro groups. Even though the mentioned factors are favorable for the retentions of analytes, the observed breakthrough volumes were small which could be caused by the small capacity of the sorbent.



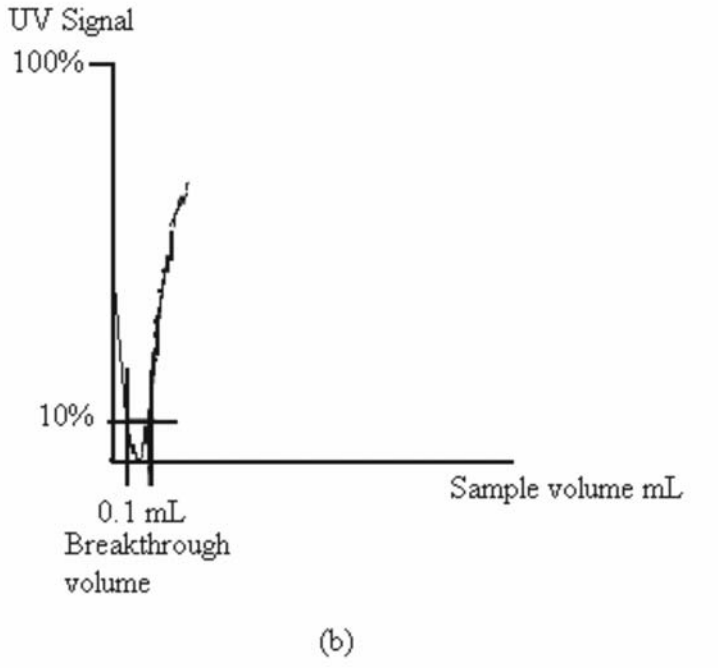


Figure 3. Breakthrough volume curve of (a) nitrobenzene and (b) 2-chlorophenol using PS-DVB as the adsorbent.

Figure 4 shows the breakthrough curve of nitrobenzene and 2-chlorophenol using PS-DVB heptadecyl ketone. Higher breakthrough volume was observed for both analytes compared to other modified PS-DVB. The breakthrough volumes for nitrobenzene was higher compared to 2-chlorophenol on PS-DVB heptadecyl ketone sorbent.

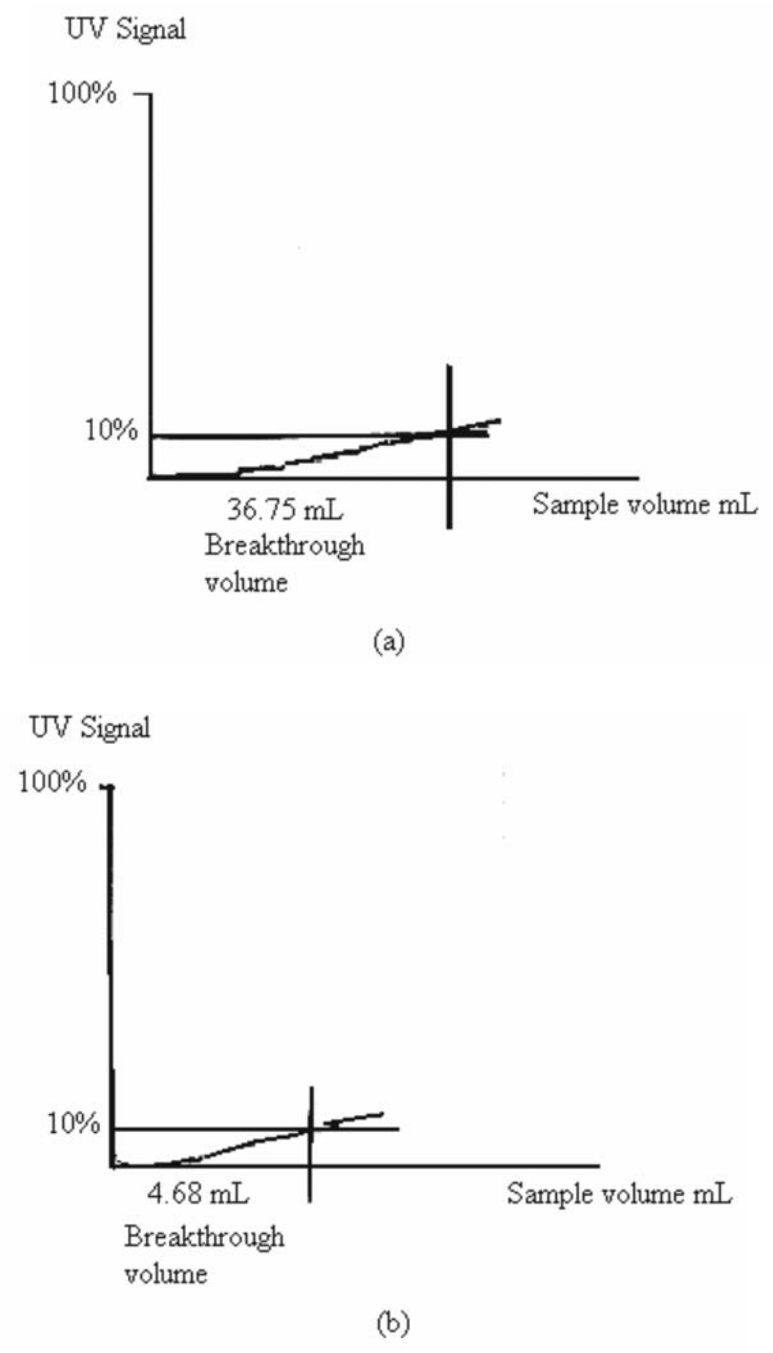


Figure 4. Breakthrough volume curves of (a) nitrobenzene and (b) 2-chlorophenol using PS-DVB heptadecyl ketone.

The presence of electron-withdrawing or positive electron resonant capacity substituents on analyte caused the polymer to donate electron to the analyte. Higher retention of 2-chlorophenol on PS-DVB heptadecyl ketone was contributed by the hydrogen bonding between the hydrogen from hydroxyl groups of the analyte and the oxygen from the carbonyl groups on the adsorbent.

According to the Lewis acid-base Theory, the benzene rings on PS-DVB and the carbonyl groups on PS-DVB heptadecyl ketone can be considered as Lewis base while the phenolic compounds can act as a Lewis acid. However, the oxygen on the carbonyl group on PS-DVB heptadecyl ketone exhibited larger dipole moment and resulted in better Lewis base property in relative to the benzene ring on PS-DVB. Consequently, the interaction of phenolic compounds was found to be much better by using PS-DVB heptadecyl ketone instead of PS-DVB as the adsorbent. The breakthrough volume for nitrobenzene and 2-chlorophenol using PS-DVB chloromethyl were 0.31 mL and 0.16 mL respectively, meanwhile for PS-DVB octadecoxy methyl were 0.12 mL and 0.10 mL respectively. The small amount of breakthrough volumes observed for the PS-DVB chloromethyl and PS-DVB octadecoxy methyl mostly is attributed to the small capacity owned by the respective sorbent, incompatibility of wetting solvent used and the homemade sorbents were unsatisfactorily synthesized.

Measurement of Percentage Recovery

The chromatogram of test compounds and internal standard obtained is given in Figure 5. All the test compounds and butyrophenone were eluted within ten minutes. The retention times for the test compounds as well as internal standard are listed in Table 3.

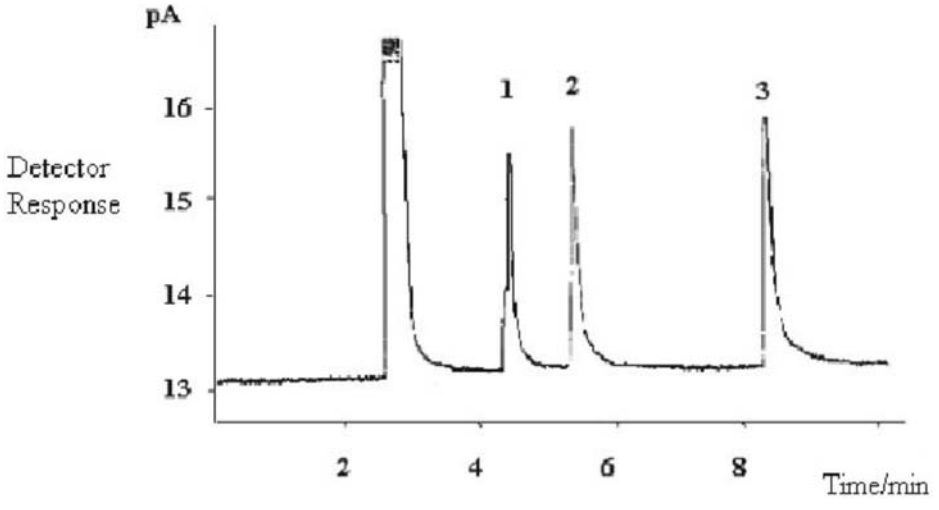


Figure 5. Separation of test compounds and butyrophenone (internal standard) using gas chromatography. Peaks: 1 – chlorophenol, 2 – nitrobenzene, 3 – butyrophenone.

The response factor is usually utilized in GC accurate quantitative calculation. In this study, 1μL of each test compounds and the internal standard stock solutions (400 ppm) were injected into the gas chromatograph to determine the response factor (F_X) for each compounds. Three injections were carried out to obtain a measure of accuracy. The response factors for each compound were calculated as per equation 3.

Table 3. Retention time of test compounds and butyrophenone in GC chromatogram.

Compound	Retention time, (t_R/minutes)
2-Chlorophenol	4.244
Nitrobenzene	5.153
Butyrophenone (Internal Standard)	7.931

The peak areas, response factors, concentrations, percentage recovery and relative standard deviations of each test compound is summarized and listed in Table 4. It was found that the recovery of the less polar compounds, nitrobenzene was higher (44%) compared to the more polar compound, 2-chlorophenol (35%).

Table 4. The average peak areas, average concentration values, percentages recovery, and relative standard deviation from the test compounds with ODS-silica SPE precolumn.

Compounds	Average Area	Average Concentration (ppm)	Recovery (%)	RSD (%)
2-Chlorophenol	4.2	139	35	7.92
Nitrobenzene	7.6	175	44	2.17

The efficiency of PS-DVB heptadecyl ketone as SPE adsorbent was evaluated by determining the analytes recovery percentage. Based on the results obtained in Table 5 it was observed that the overall results demonstrated high recoveries in the range of 68%-110% with RSD values of 2.07% to 2.18% using the PS-DVB heptadecyl ketone as an adsorbent. This adsorbent shows a better affinity compared to ODS-silica with large difference between the percentage recovery of the analyte. A flow rate of 0.1 mL/min was used to ensure sufficient interaction between the analytes and elution solvent, which aids the mass transfer of analytes from sorbent to elution solvent. The PS-DVB heptadecyl ketone exhibited strong hydrophobic characteristics due to the polymer backbone and hydrophilic properties through the polar carbonyl functional group on its surface.

Table 5. Average peak areas, average concentration values, percentage of recovery, and relative standard deviation for test compounds desorbed from PS-DVB heptadecyl ketone.

Compounds	Average Area	Average Concentration (ppm)	Recovery (%)	RSD (%)
2-Chlorophenol	7.45	273	68	2.18
Nitrobenzene	17.8	441	110	2.07

The analytes were not detected during the recoveries study using commercial PS-DVB and other modified PS-DVB. This might be due to the small adsorbent capacity to trap the analytes. The analytes desorbed from the sorbent was probably too low to be detected by GC FID. The capacity of the adsorbent can be increased by increasing the sorbent mass, but in this study, a fix volume of precolumn was used to pack the sorbents.

The efficiency of ODS-silica and PS-DVB heptadecyl ketone in SPE stailess steel precolumn was compared by means of percentage recovery of test compounds. Table 6 shows the comparison of recovery percentage and RSD values obtained using ODS-silica and PS-DVB heptadecyl ketone as adsorbents.

The higher recoveries of the test compounds on PS-DVB heptadecyl ketone were due to the hydrophilic character of the introduced functional groups which increases its surface polarity and improved the sorbent wetting property. The ability of polar surface to reduce the surface tension of the water thus enabled the aqueous sample to interact with the resin surface and enhanced the mass transfer of the analytes from the water solution to the sorbents. Although PS-DVB heptadecyl ketone has a hydrophobic surface, it also contains relatively large number of active aromatic sites, which allow π-π interactions between aromatic analytes and the sorbents.

Table 6. Comparison of percentages of recovery (%R) and relative standard deviation for the extraction of test compounds using ODS-silica and PS-DVB heptadecyl ketone.

	ODS-silica		PS-DVB heptadecyl ketone	
Compound	**% R**	**RSD %**	**% R**	**RSD %**
2-Chlorophenol	35	7.92	68	2.18
Nitrobenzene	44	2.17	110	2.07

Even though nitrobenzene is less polar compared to 2-chlorophenol, greater amount of nitrobenzene was eluted by methanol due to amount of nitrobenzene retained on the sorbent was very high. The low recoveries of ODS-silica might be due to the hydrophobic surface of the sorbent. The consequence is poor surface contact with predominantly aqueous solutions. Less analyte is retained on the sorbent and therefore less analyte desorbed when eluting with methanol.

In this experiment, methanol was chosen as the elution solvent because its volatile characteristic was compatible to the subsequent gas chromatography analysis. Methanol was found to be a good elution solvent for the extraction of polar analytes using reversed phase adsorbents. The hydroxyl group on methanol that contributed to its polarity enables the solubility of analytes that were retained on the adsorbent.

CONCLUSIONS

Three adsorbents were studied and ODS-silica was used as comparison. Based on this study, PS-DVB heptadecyl ketone adsorbent exhibited higher breakthrough volume of 36.75 mL and 4.68 mL for nitrobenzene and 2-chlorophenol respectively with % RSD at 2.69% and 21.52% compared to the other modified PS-DVB.

It was established that the PS-DVB heptadecyl ketone has a very good adsorbent capacity compared to other modified PS-DVB and comparable to that of commercial ODS-silica. As for percentage recovery, the PS-DVB heptadecyl ketone, shows high recoveries, 110% for nitrobenzene and 68% for 2-chlorophenol as well as good reproducibility with relative standard deviation between 2.07% and 2.18%.

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GASTROINTESTINAL PROTOZOAN PARASITES AMONGST SCHOOLCHILDREN IN INANAM, SABAH

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ABSTRACT. *Intestinal parasitosis is still an important public health problem. The aim of this study was to determine the prevalence of gastrointestinal protozoan parasites (GIP) in schoolchilden and its association with socio-economic and environmental factors. A series of sample collections for stool was carried out in Sekolah Kebangsaan Inanam II, Kota Kinabalu, Sabah. Samples from 100 schoolchildren were examined by direct smear and formol-ether concentration techniques. The modified Kato-Katz technique was performed to estimate the parasitic burden, expressed in the number of protozoa per gram of stool. The proportion of overall infected samples was 31%. When ranked by proportion, parasite loads were found as follows: Entamoeba histolytica (83.87%), Giardia lamblia (35.48%), Entamoeba coli (22.58%), Entamoeba hartmanni (25.81%), Iodamoeba butschlii (19.35%) and Endolimax nana (6.45%). Both single and double infections in the study had equal percentages (35.48%), followed triple infection (29.03%). There were no significant effects of protozoan infection on weight, height, attendance to school and examination results of the schoolchildren (Independent Group t-Test; $p > 0.05$). No significant association were found between the protozoan infection and the socio-economic and environmental factors (gender, age, occupation status of mother, house area category and the degree of household crowding). We conclude that the parasitic burden amongst the schoolchildren of Sekolah Kebangsaan Inanam II is minimal and is of less concern.*

KEYWORDS. Gastrointestinal, protozoan infection, *Entamoeba histolytica*, *Entamoeba coli*, *Giardia lamblia*

INTRODUCTION

Intestinal parasitic infection is still an important public health problem in underdeveloped or developing countries. It is known to be affected by several factors including personal hygiene, dietary habits, education levels of the community, socioeconomic status, climate and other environmental factors. Intestinal parasites are more frequently encountered during schooling age, most likely linked to relatively less developed hygienic habits. When burden in children is pronounced intestinal parasites might cause serious health conditions and problems notably diarrhea, malnutrition, malabsorption, mental retardation and even death.

Parasitic helminths and protozoans are still a public health concern in Malaysia, although are well controlled and only sporadically limited to specific areas or within certain population groups e.g. the aboriginal settlements and amongst people living in remote areas (MOH Malaysia, 2008). The studies on the prevalence of protozoan parasites have been carried out mainly amongst schoolchildren in Peninsular Malaysia (Noorhayati *et al.*, 1981; Hamimah *et al.*, 1982; Sinniah & Rajeswari, 1988). Little or no work has been done in Malaysian Borneo.

The main purpose of this study was to determine the prevalence of gastrointestinal protozoan parasites (GIP) among schoolchilden and to see its impacts on physical and their learning abilities. Acknowledging that gastrointestinal infections by endoparasites are common, the levels infections that retrogressively affect the mental and physical growth of children must not be taken lightly. We present here the case study in Sekolah Kebangsaan Inanam II, a sub-urban school immidiately outside the Kota Kinabalu City.

MATERIALS AND METHODS

This study was performed in SK Inanam II, which is located in Inanam about 15 kilometers from Kota Kinabalu, capital city of Sabah, Malaysia. Sabah has a tropical climate where the temperature arise around 30-40^{o}C. Sanitary conditions are sufficient in large parts of the city. The stool samples of 100 children aged 7 – 9 years (51% female and 49% male) were obtained through consent from September 2005 to January 2006.

The stool samples were firstly examined through direct smear or saline-iodine method. The size of the detected parasite was evaluated by ocular micrometric method. Formol – ether concentration method was then performed to enhance detections of cysts. Amoeba trophozoites were observed in fresh smears. To estimate the protozoa burden, modified Kato-Katz procedure was performed by calculating the number of cysts or trophozoites in a gram of stool.

Variables used to measure the mental and physical impacts of infections were weight, height, attendance to school, and academic achievements (here translated as the results of examinations). The following parameters were recorded through questionnaires to evaluate the associations of socio-economic and the environmental factors with infection rates: gender, age, occupation status of mother, house area category and degree of crowding. Consent was obtained from parents prior to sample collections.

The independence groups t-test was used to examine the difference between infected and uninfected group of schoolchildren. The association between any two types of variables in this study were examined through chi-square test. Data were evaluated by SPSS for Windows (version 12.0) and all statistics were set to the significance level of $p<0.05$.

RESULTS

Out of the 100 stool samples examined, 31 of them (31%) were positive for GIP. Among those infected with GIP (see Table 1), 83.87% of the schoolchildren were infected with *Entamoeba histolytica*, followed by *Giardia lamblia* (35.48%), *Entamoeba hartmanni* (25.81%), *Entamoeba coli* (22.58), *Iodamoeba butschlii* (19.35%) and *Endolimax nana* (6.45%).

Table 1. Distribution of positive cases according to the parasites species.

Parasites	N=100	%
Entamoeba histolytica	26	83.87
Giardia lamblia	11	35.48
Entamoeba hartmanni	8	25.81
Entamoeba coli	7	22.58
Iodamoeba butschlii	6	19.35
Endolimax nana	2	6.45

More than a third of the schoolchildren were infected with both single species and double species infection of protozoa (35.48%), followed by the triple infection (29.03%). Table 2 shows that among the protozoa infectants, infection with *Entamoeba histolytica* was the commonest type of infection among infected subjects followed by *Entamoeba coli* and *Iodamoeba butschlii*. Oddly, triple infections by protozoa species were proportionally high in the infected group i.e. 29.03% (Table 2).

Table 2. Distribution of positive cases according to the parasites species.

GIP parasites species	No. +ve	%
Single infection	**11**	**35.48**
Entamoeba histolytica	7	22.58
Entamoeba coli	2	6.45
Iodamoeba butschlii	2	6.45
Double infections	**11**	**35.48**
Entamoeba histolytica + *Entamoeba hartmanni*	4	12.90
Entamoeba histolytica + *Giardia lamblia*	4	12.90
Entamoeba histolytica + *Entamoeba coli*	1	3.23
Entamoeba histolytica + *Iodamoeba butschlii*	1	3.23
Entamoeba histolytica + *Endolimax nana*	1	3.23
Triple infections	**9**	**29.03**
Entamoeba histolytica + *Giardia lamblia* + *Entamoeba coli*	3	9.68
Entamoeba histolytica + *Giardia lamblia* + *Iodamoeba butschlii*	3	9.68
Entamoeba histolytica + *Entamoeba hartmanni* + *Endolimax nana*	1	3.23
Entamoeba histolytica + *Entamoeba hartmanni* + *Entamoeba coli*	1	3.23
Entamoeba histolytica + *Entamoeba hartmanni* + *Giardia lamblia*	1	3.23
Total positive for GIP parasites	**31**	**100.00**

The quantitative method of discriminating GIP infections in this study was performed with modifications to the procedure for helminth as suggested in Suzuki, 1975 and Belizario *et al.*, 2001. This method calculated the number of protozoa (cysts or trophozoites) that are present in a gram of stool sample. Infection burdens were classified as light (1 to 100), moderate (101 to 1000) and heavy (>1000) protozoa in a gram of stool. No heavy infections were observed in this study; the number of moderately burdened children dropped steeply from the number of the ligthly burdened group by a factor of 4 (see Table 3).

Table 3. Level of GIP infection/burden among schoolchildren.

GIP burden	No. of samples	Percentage
Light	25	80.65%
Medium	6	19.35%
Heavy	-	-
Total	31	100.00%

The association of Gender and Age to GIP infection

Infection was higher in females (Table 4) despite the very close similarity of the gender proportions.

Table 4. The GIP infection between gender.

	GIP Infection				Total	
	Positive		Negative			
Gender	Count	Percentage	Count	Percentage	Count	Percentage
Male	11	35.5%	38	55.1%	49	49.0%
Female	20	64.5%	31	44.9%	51	51.0%
Total	31	100.0%	69	100.0%	100	100.0%

There was no difference between infection burdens between age, the results demonstrated stable rates between age. Table 5 shows the distribution of GIP infection among schoolchildren aged 7, 8, and 9 years old.

Table 5. The GIP infection among ages.

	GIP Infection				Total	
	Positive		Negative			
Age	Count	Percentage	Count	Percentage	Count	Percentage
7	11	35.5%	22	31.9%	33	33.0%
8	9	29.0%	25	36.2%	34	34.0%
9	11	35.5%	22	31.9%	33	33.0%
Total	31	100.0%	69	100.0%	100	100.0%

The statistical analysis result showed that there was no significant association between GIP infection and gender ($\chi^2 = 3.284$, df = 1, $p > 0.05$). There was also no significant association between GIP infection and age ($\chi^2 = 0.494$, df = 2, $p > 0.05$).

Effect of GIP infection on mental and physical factors

Infections, in general, have no impact on the mental and physical conditions of the schoolchildren involved in this study. The descriptive statistics of GIP infection (Table 6) shows that the means of negative infection were lower than positive infection in all variables.

Table 6. The GIP infection on weight, height, attendance to school and examination result.

Factors	GIP Infection	N	Mean	Std. Deviation	Std. Error Mean
Weight	Positive	31	21.0	6.0	1.0
	Negative	69	20.8	4.9	0.5
Height	Positive	31	121.6	7.6	1.3
	Negative	69	121.9	6.7	0.8
Attendance to school	Positive	31	94.7	4.2	0.7
	Negative	69	93.6	5.5	0.6
Examination result	Positive	31	53.7	24.2	4.3
	Negative	69	48.1	23.9	2.8

The results showed no significant difference for the means of GIP infections on weight of schoolchildren ($t = 0.208$, $df = 98$, $p > 0.05$), height ($t = -0.230$, $df = 98$, $p > 0.05$), attendance to school ($t = 1.009$, $df = 98$, dan $p > 0.05$), examination result ($t = 1.072$, $df = 98$, $p > 0.05$). Thus suggests that the infections are within the tolerable levels by the children.

The association between GIP infection and other factors (socio-economic and the environmental factors)

The crosstabulation tables details for other factors (socio-economic and the environmental factors) are shown in Table 7. The was no observably significant association between GIP infection and other factors. The value obtained for occupation status of mother ($\chi^2 = 1.185$, $df = 1$, $p > 0.05$), house area category ($\chi^2 = 5.168$, $df = 2$, $p > 0.05$), and degree of crowding ($\chi^2 = 0.817$, $df = 2$, $p > 0.05$).

Table 7. The crosstabulation tables of: a) occupation status of mother, b) house area category, and c) degree of crowding, with GIP infection.

a) Occupation status of mother * GIP Infection Crosstabulation

Occupation status of mother	GIP Infection				Total	
	Positive		Negative			
	Count	Percentage	Count	Percentage	Count	Percentage
Work	5	17.2%	18	27.7%	23	24.5%
Housewife	24	82.8%	47	72.3%	71	75.5%
Total	29	100.0%	65	100.0%	94	100.0%

b) House area category * GIP Infection Crosstabulation

House area Category	GIP Infection				Total	
	Positive		Negative			
	Count	Percentage	Count	Percentage	Count	Percentage
Rural	15	53.6%	34	50.0%	49	51.0%
Sub-rural	9	32.1%	11	16.2%	20	20.8%
Urban	4	14.3%	23	33.8%	27	28.1%
Total	28	100.0%	68	100.0%	96	100.0%

c) Degree of crowding * GIP Infection Crosstabulation

Degree of crowding	GIP Infection				Total	
	Positive		Negative			
	Count	Percentage	Count	Percentage	Count	Percentage
4-6	14	45.2%	22	35.5%	36	38.7%
7-9	9	29.0%	21	33.9%	30	32.3%
10 >	8	25.8%	19	30.6%	27	29.0%
Jumlah	31	100.0%	62	100.0%	93	100.0%

DISCUSSION AND CONCLUSION

Apparently, the number of infected schoolchildren in this study was not small taking into account the location of this school which is close to the State Capital. However, the infecting species are common and are well documented i.e. *Entamoeba histolytica*, *Giardia lamblia*, *Entamoeba hartmanni*, *Entamoeba coli*, *Iodamoeba butschlii* and *Endolimax nana* (Hamimah *et al.,* 1982; Noorhayati *et al.*, 1981; Sinniah & Rajeswari, 1988). *Entamoeba histolytica* represented the highest proportion similar to the findings of previous workers e.g. Noorhayati *et al.* (1981) and Sinniah & Rajeswari (1988).

The levels of infection or GIP burden only showed light and moderate groups with the heavy group absent; mostly were single and infection, followed by triple infection. The result was similar to Hamimah *et al.* (1982) in Hospital Kuala Lumpur which stated that the amount of single and double infection was higher than triple infection. The triple infection group was, nevertheless, higher as compared to previous studies. In theory there should be smaller number of children harboring three or more species of endoparasites, acknowledging the natural tolerance of human being to parasites or the natural difference of surviving rates of different species parasites in human alimentary tracts. What this suggests would either be that there were higher possibilities of exposures to multiple species infections or that the natural tolerance of schoolchildren in this study was somewhat compromised by unknown factors.

The school environment can potentially be the medium in the spreading protozoa, which include water, food, and mouth-to-anus cycle – the last medium is known to be a common habit of young children (Boreham *et al.*, 1990) especially in a less sanitarily-trained children (Pang, 1989). Protozoan cysts may infect human through drinking of contaminated water and through eating improperly-prepared foods.

Especially pronounced infection rates have been proven to have affected children's growth and mental development (Pang, 1989; Thomas, 1983). This was not seen in this study, demonstrating that infections rates observed here were still within the natural tolerance levels by the children. Interestingly, we have not obseved outstanding differences career and non-career mothers but the rates of infection did not reflect the common opinion. Therefore, the common perception that working mothers give less attention to their children and consequently increases potentials for schoolchildren to get infected is not exactly true. Infection can be prevented as long as the career mothers care about their children's hygiene. Schoolchildren, either in rural or urban areas, regardless the number of members in their families, still carry risks of infection if hygienic awareness are neglected (Boreham *et al.*, 1990; Parmar, 1995).

GIP, clearly, has not been eradicated, even in sub-urban areas – in this case in a community living close to a state capital. This case showed that although eradication has not taken place, the standard of living and the awareness on hygene are not in apalling states. Nevertheless, it should never become as an excuse not to provide proper public facilities to reduce the infection rates further.

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PROCEDURES IN GETTING BEST MODEL USING MULTIPLE REGRESSION

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ABSTRACT. *This work illustrated the procedures in getting the best model using Multiple Regression. Multiple Regression models were involved up to the higher-order interaction and all the possible models were considered. The possible models were reduced to selected models using progressive elimination of variable with the highest p-value (coefficient test). To enhance the understanding of the whole concept in this work, Multiple Regression with eight selection criteria (8SC) had been explored and presented in the process of getting the best model from selected models. In this work, a numerical illustration had been included to get a clear picture of the process of getting the best model. In conclusion, the best model could be obtained by using Multiple Regression.*

KEYWORDS. Multiple regressions, higher-order interaction variables, eight selection criteria (8SC), progressive elimination of variables

INTRODUCTION

Regression analysis is a statistical methodology that utilises the relation between two or more quantitative variables in order to predict the dependent variable. It is one of the most flexible and widely used techniques of quantitative analysis. Regression analysis is widely used in business, the social and behavioural sciences, the biological sciences and many other fields. Regression analysis allows the researchers to estimate the relative importance of independent variables in influencing a dependent variable. Regression analysis also help researcher to identify a mathematical equation which describes the relation between the independent variables and the dependent variable.

Multiple Regression is the extension of simple regression. Multiple Regression models are used when economic theory suggested that the prediction of dependent variable can be made more accurate by using more independent variables. Multiple Regression is a technique that predicts the effect on the average level of the dependent variable of a unit change in any independent variable while the other independent variables are held constant.

THE PROCEDURES

A multiple regression is an extension of the simple linear regression analysis. Simple regression analysis could analyse a relationship between a dependent variable with a single independent variable. The same idea was utilised to analyse relationship between a dependent variable with two or more independent variables. Consider a model (1) with seven independent variables and a constant.

$$Y = \gamma_0 + \gamma_1 W_1 + \gamma_2 W_2 + \gamma_3 W_3 + \gamma_{12} W_{12} + \gamma_{13} W_{13} + \gamma_{23} W_{23} + \gamma_{123} W_{123} + u \qquad (1)$$

The variables W_1, W_2 and W_3 are single independent variables. The variables W_{12}, W_{13}, W_{23} and W_{123} are called as interaction variables (first-order and second-order interaction variables, respectively) which are the product of corresponding single independent variables as indicated by the subscript of individual single digit at each interaction variable. Thus model (1) can be expressed as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 + u \qquad (2)$$

Where β_0 is the constant term of the model, β_j is the coefficient of the corresponding variable X_j for j=1, 2, ..., k. The model (2) can be extended and expressed in a general form as shown in model (3). Therefore, in this work, the general Multiple Regression model is written in the following format

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \ldots + \beta_k X_{ki} + u_i \qquad (3)$$

where Y_i is the *i*th value of the considered dependent variable Y, β_j is the coefficients that describes the corresponding variable X_j for j=1, 2, ..., k and X_{ji} is the *i*th value of independent variable X_j where i = 1, 2, …, n. and j=1,2,…,k. The u_i is the corresponding error term of the *i*th observation. Therefore, $X_{1i,} X_{2i}$, ..., X_{ki} are capable of providing a better prediction of the value *Y* where *k+1* is the number of parameters of the model (3). Thus, the basic assumptions of multiple regression models are made on the error terms *u* and the values of independent variables $X_1, X_2, ..., X_k$ as follows (Gujarati, 2006):

Table 1. The Basic Multiple Regression Assumptions.

	Name	Description	Multiple Regression
1	Linearity of Model	Linear	Linear in the unknown parameters
2	Some of the observed data are different	Var(independent variable) > 0	At least one of the observations in each independent variable is different
3	Conditional mean of u, given X, is zero	u_t is a random variable	$E(u\| X_1, X_2, \ldots, X_k) = 0$
4	X are given and hence can be treated as non random	$Cov(X_s, u_t) = 0$	Each of the independent variable is uncorrelated with u
5	Homoscedasticity	$Var(u_t\| X_1, X_2, \ldots, X_k) = \sigma^2$	Given $X_1, X_2, \ldots, X_k$, u has a constant variance
6	Serial independence	$Cov(u_s, u_t X_1, X_2, \ldots, X_k) = 0$	Given $X_1, X_2, \ldots, X_k$, u_s and u_t are independently distributed for all $s \neq t$
7	Sample size is greater than number of parameters	n > k	Number of observations (n) is greater than the number of estimated parameters (k), n> k.
8	Normality of Errors	Error term is normally distributed	Given $X_1, X_2, \ldots, X_k$, u_t is normally distributed

In the development of the Multiple Regression model, there are four phases involved. The phases are possible models, selected models, best model and goodness-of-fit test. The phases are explained in detail in the following sections.

All Possible Models

The coefficients of the model will be estimated using ordinary least square (OLS) method. Before the analysis was carried out, the entire possible models up to higher-order interaction must be listed out and considered. This is to help in determining the significant variables that contribute to the dependent variable. The number of possible models can be calculated as follows:

$$N = \sum_{j=1}^{k} j({}^{k}C_{j}) \qquad (4)$$

where N is number of possible models and k is number of variables. For example, when $k = 3$ the possible models are $1({}^{3}C_{1}) + 2({}^{3}C_{2}) + 3({}^{3}C_{3}) = 12$.

Selected Models

Let's consider a model defined in (3) with k variables (with k+1 number of parameters which include a constant term) as one of the possible models. In the process of getting a selected model from a possible model, global test, coefficient test (eliminating insignificant variables) and Wald test should be carried out in order to get a set of significant variables which contribute to the dependent variable, Y. Set a level of significant, α at 5% for all the tests that carried out. The global test is carried out to investigate whether it is possible for all the independent variables in the model defined to have zero net regression coefficients (Lind *et al*., 2005). The hypothesis for global test is as follows:

H_o: all the coefficients β's are zero

H_1: at least one β's is nonzero

Table 2. ANOVA Table for Global Test

Source of variations	Sum of Squares	*df*	Mean Sum of Square	F
Regression	$\sum_{i=1}^{n}(\hat{Y}_i - \overline{Y})^2$	k	MSR	$F=\frac{MSR}{MSE}$
Residual	$\sum_{i=1}^{n}(Y_i - \hat{Y}_i)^2$	n-k-1	MSE	
Total	$\sum_{i=1}^{n}(Y_i - \overline{Y})^2$	n-1		

The Table 2 shows process getting the F_{cal} where F_{table} is $F_{\alpha,k,(n-k-1)}$ taken from an F table. The decision is to reject the null hypothesis where all the regression coefficients are zero if F_{cal} is greater than F_{table}. The global test is carried out for all the possible models arised. The next step is to perform the coefficient test for all the coefficients in the model. This is to test the coefficient of the corresponding variable with the value of zero (Lind *et al*., 2005). The hypothesis for this j^{th} coefficient is as follows:

$H_o: \beta_j = 0$

$H_1: \beta_j \neq 0$ for $j = 1, 2, \ldots, k$

The $t_{cal} = \left[\hat{\beta}_j - \beta_j(H_0)\right] / se(\hat{\beta}_j)$ and the $t_{critical}$ is $t_{\alpha/2,(n-k-1)}$ where se ($\hat{\beta}$) is the standard error of $\hat{\beta}$ and $_j(H_o)$ is the corresponding value of $_j$ under H_o. The decision is to reject the null hypothesis where regression coefficient is zero if $|t_{cal}|$ is greater than $|t_{table}|$. Similar procedure is carried out for other coefficients where the test leads to the elimination of variable (highest p-value > 0.05). The elimination process is as follows:

a. Drop the independent variable with the smallest $|t_{cal}|$ (and less than $|t_{critical}|$) or near to zero and then rerun
b. If there are still regression coefficients that are not significant, drop the variable with the smallest $|t_{cal}|$ (and less than $|t_{critical}|$) or near to zero again
c. Repeat steps (a) and (b) until there is no more $|t_{cal}|$ near to zero

To get a clear picture of the elimination procedure, consider the model (3) with k+1 parameters. If number of parameters (k+1) is greater than sample size (n), then remove the model from the analysis. For models with (k+1) < n the following steps are carried out:

Step 1: Obtain matrix $\boldsymbol{X}$ and vector $\boldsymbol{Y}$. Then, obtain $\boldsymbol{X}^T\boldsymbol{X}$, $(\boldsymbol{X}^T\boldsymbol{X})^{-1}$ and $\mathbf{X}^T\mathbf{Y}$.
Step 2: Based on OLS estimation, obtain $\hat{\boldsymbol{\beta}} = (\boldsymbol{X}^T\boldsymbol{X})^{-1}\,\mathbf{X}^T\mathbf{Y}$. Therefore, $\hat{\boldsymbol{Y}} = \boldsymbol{X}^T\hat{\boldsymbol{\beta}}$.
Step 3: Obtain the residuals, sum of square errors (=SSE) and mean sum of square errors (=MSE)

3.1 Residual $= Y_i - \hat{Y}_i$ for i=1,2,...,n

3.2 $SSE = \sum_{i=1}^{n} (Y_i - \hat{Y}_i)^2$ and df = n-k-1

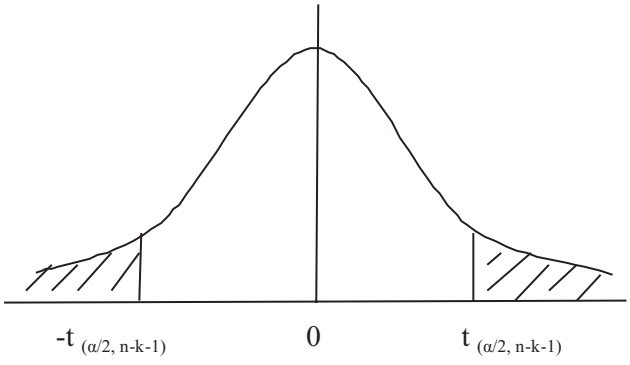
3.3 $MSE = \dfrac{\sum_{i=1}^{n} (Y_i - \hat{Y}_i)^2}{n-k-1}$, $\hat{\sigma}^2 = MSE$

Step 4: Obtain var($\hat{\beta}$) where var($\hat{\beta}$) = $\hat{\sigma}^2(\boldsymbol{X}^T\boldsymbol{X})^{-1}$

Step 5: Calculate the t_{cal} for each β's where

$$t_j = \frac{\hat{\beta}_j - \beta(H_0)}{se(\hat{\beta}_j)} \qquad j = 1, 2, ..., k$$

Step 6: Obtain $t_{critical}$ from t table where the value is $t_{\alpha/2,(n-k-1)}$



Step 7: Let t* be the minimum $\{t_1, t_2, ..., t_k\}$. If $|t^*| < |t_{critical}|$ or $|t^*| \rightarrow 0$, eliminate the corresponding independent variable.

Step 8: Repeat the step 1 to Step 7 until there is no more $|t_{cal}| < |t_{critical}|$. Otherwise, the selected model is achieved.

The omission of the variable is carried out one by one. Immediately after the completion of coefficient test where the insignificant variables were removed, a Wald test is carried out to justify the removal (Ramanathan, 2002). In the Wald test, the restricted model is the selected model whereas the unrestricted model is the initial possible model considered. Consider the following situation where:

Unrestricted model (a possible model):

U: $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_m X_m + \beta_{m+1} X_{m+1} + \ldots + \beta_k X_k + u$

Obtain SSE(U) and df(U) = n-k-1.

Restricted model (selected model):

R: $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_m X_m + r$

Obtain SSE(R) and df(R)= n-m-1

The hypothesis to be tested:

H_0: $\beta_{m+1} = \beta_{m+2} = \ldots = \beta_k = 0$

H_1: At least one $\beta's$ is nonzero

with the corresponding ANOVA table shown in Table 3.

Table 3. ANOVA for Wald Test

Source of variations	Sum of Squares	*df*	Mean Sum of Squares	F
Differences (R-U)	SSE(R) – SSE(U)	m-k	$\frac{[SSE(R) - SSE(U)]}{m - k}$	$F = \frac{[SSE(R) - SSE(U)]/[DF(R) - DF(U)]}{SSE(U)/DF(U)}$
Unrestricted (U)	SSE(U)	n-m-l	SSE(U) / (n-m-l)	
Restricted (R)	SSE(R)	n-k-l		

The Table 3 shows the F_{cal} for Wald Test and the F_{table} is $F(DF_R - DF_U, DF_U, \alpha)$ which is equal to F(m-k, n-m-1, α). The decision is to reject the null hypothesis if F_{cal} is greater than F_{table}. Similar procedure of Wald test is carried out for all the selected models.

Best Models

The best model will be selected from selected models that are obtained from previous test that had been conducted in Section 2.2. The best model will be identified with the aid of the eight selection criteria (8SC). Recently, several criteria have been proposed in literatures in order to choose a best model. Ramanathan (2002) has reviewed many of the criteria where these criteria take the form of the sum of square error (SSE) multiplied by a penalty factor that depends on complexity of the model considered. A more complex model will reduce SSE but raise the penalty value. A model with a lower value of a criterion statistics is judged to be preferable.

The model selection criteria considered in this work are finite prediction error (FPE), Akaike information criterion (AIC), Hannan and Quinn criterion (HQ criterion), SCHWARZ (Schwarz, 1978), SHIBATA (Shibata, 1981) and RICE (Rice, 1984), generalised cross validation (GCV) developed by Golub *et al.* (1979) and SGMASQ. Finite prediction error (FPE) and Akaike information criterion (AIC) were developed by Akaike (1970, 1974). HQ criterion was suggested by Hannan and Quinn (1979). Table 4 shows the details of each model selection criteria.

Table 4. Model Selection Criteria

EIGHT SELECTION CRITERIA (8SC)	
AIC: $\left(\frac{SSE}{n-k}\right)(e)^{2(k+1)/n}$ **(Akaike, 1970)**	**RICE:** $\left(\frac{SSE}{n-k}\right)(e)^{2(k+1)/n}$ **(Rice, 1984)**
FPE: $\left(\frac{SSE}{n-k}\right)\frac{n+k+1}{n-(k+1)}$ **(Akaike, 1974)**	**SCHWARZ:** $\left(\frac{SSE}{n-k}\right)(n)^{2(k+1)/n}$ **(Schwarz, 1978)**
GCV: $\left(\frac{SSE}{n-k}\right)\left[1-\frac{k+1}{n}\right]^{-2}$ **(Golub *et al.*, 1979)**	**SGMASQ:** $\left(\frac{SSE}{n-k}\right)\left[1-\frac{k+1}{n}\right]^{-1}$ **(Ramanthan.2002)**
HQ: $\left(\frac{SSE}{n-k}\right)(\ln n)^{2(k+1)/n}$ **(Hannan and Quinn, 1979)**	**SHIBATA:** $\left(\frac{SSE}{n-k}\right)\frac{n+2(k+1)}{n}$ **(Shibata, 1981)**

SSE is the sum of square error, $k+1$ is the number of estimated parameters and n stands for sample size. There is a condition to be fulfilled when using these model selection criteria, that is $2(k+1) < n$.

Goodness-of-fit Test

Once the best model had been obtained, the residual analysis was carried out to investigate if the resulting residual is random and normally distributed. Residual analysis should be carried out on the best model to verify whether the residuals are random and normally distributed. Ismail *et al.* (2007) stated that randomness test should be carried out to investigate the randomness of residual. One of the MR assumptions is the error term should follow a normal distribution. Besides that, Kolmogorov-Smirnov test is used to check the normality assumption of the residuals. Scatter plot, histogram and box-plot of the residuals are to a get a clear picture of distribution of the residual. These plots are used as supporting evidence besides the two quantitative tests.

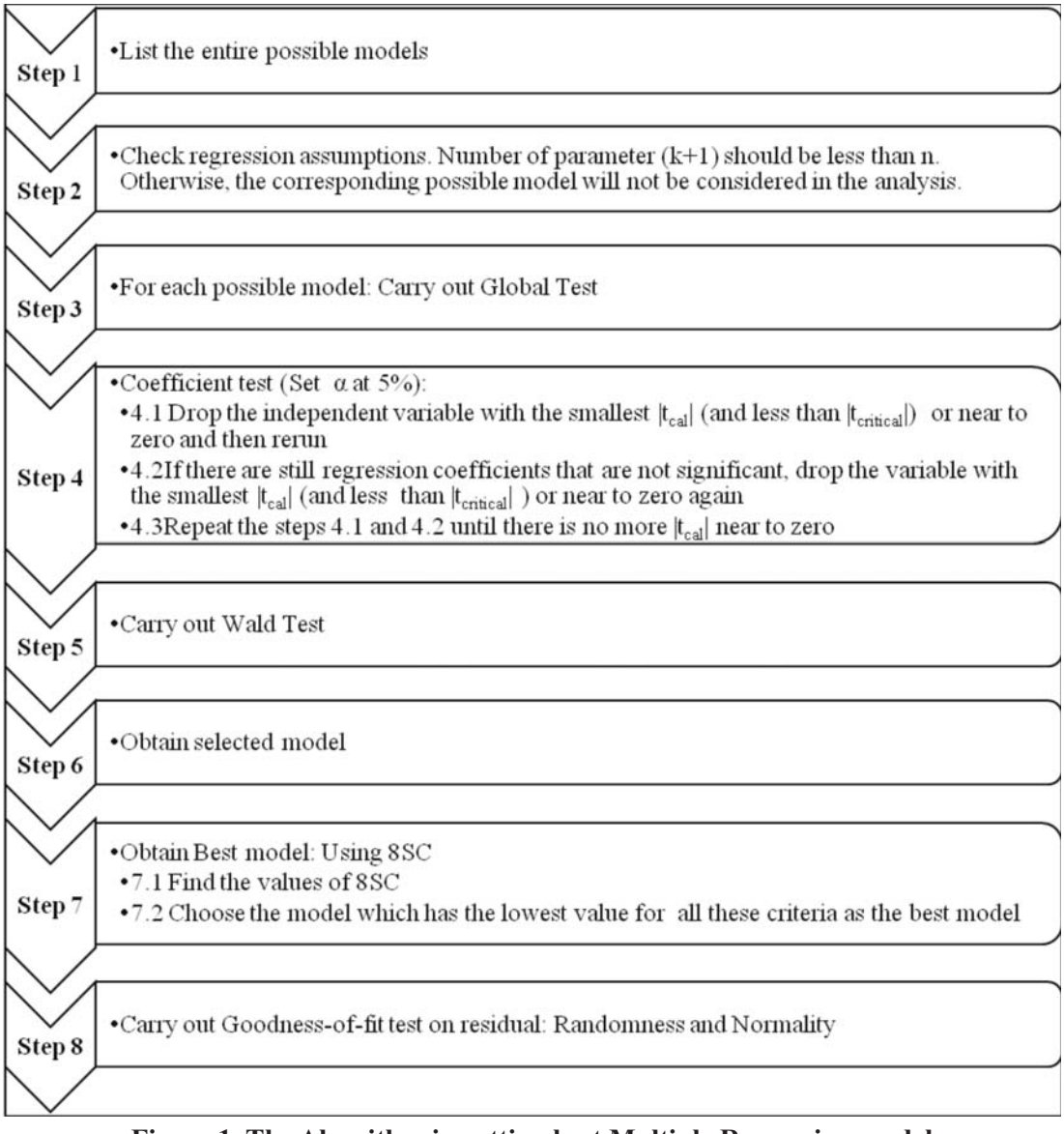


Figure 1. The Algorithm in getting best Multiple Regression model

The whole procedures in getting a best Multiple Regression model are summarized in eight simplest steps as shown in Figure 1.

NUMERICAL ILLUSTRATION

Thus, in order to get a clear picture of the procedures in getting a best model, a numerical illustration is included in this work. The data used in this work is collected in Oxford, Ohio in 1988. In this work, we are relating the selling price (Y) of a house to its selected characteristics such as floor area in square feet (X_1), number of rooms (X_2), number of bedrooms (X_3), the age in years of the house (X_4) and number of bathrooms (X_5). We analyse the contribution of a set of selected attributes in determining the selling price of 63 single-family residences sold during 1988 in Oxford, Ohio.

Table 4 shows the relationship between selling price of a house and its selected attributes. There is a significant positive relationship (correlation coefficient, r) between selling price and floor area, indicating that selling price increases as the floor area increases ($r = 0.785$, p-value < 0.0001). There is a significant positive relationship also between selling price and number of rooms, that is the selling price increases as the number of rooms increase ($r= 0.580$, p-value < 0.001). The relationship between selling price and number of bedrooms is also significant and positive relationship ($r = 0.512$, p-value < 0.001). Selling price and number of bathrooms has a significant positive relationship where selling price increases as the number of bathrooms increase ($r = 0.651$, p-value < 0.001). A negative relationship is commonly reflected between selling price and the age of the house ($r= -0.289$, p-value <0.05) where the selling price is decreased as the age of the increased.

Table 5. The Pearson correlation for house selling price and its characteristics.

	Y	**X_1**	**X_2**	**X_3**	**X_4**	**X_5**
Y	1	0.785(**) .000	0.580(**) .000	0.512(**) .000	-0.289(*) 0.021	0.651(**) 0.000
X_1	0.785(**) 0.000	1	0.711(**) 0.000	0.754(**) 0.000	-0.109 0.395	0.628(**) 0.000
X_2	0.580(**) 0.000	0.711(**) 0.000	1	0.722(**) 0.000	0.170 0.183	0.402(**) 0.001
X_3	0.512(**) 0.000	0.754(**) 0.000	0.722(**) 0.000	1	0.017 0.893	0.352(**) 0.005
X_4	-0.289(*) 0.021	-.0109 0.395	0.170 0.183	.017 .893	1	0.409(**) 0.001
X_5	0.651(**) 0.000	0.628(**) 0.000	0.402(**) 0.001	0.352(**) 0.005	0.409(**) 0.001	1

** Correlation is significant at the 0.01 level and * Correlation is significant at the 0.05 level.

As can be seen from the highlighted triangle in Table 5, there is no existence of multicollinearity (|correlation coefficient| > 0.95) between the independent variables. According to Hocking (2003), multicollinearity exists when correlation coefficient| > 0.95. Thus, no further treatment or modification is required on the given data. The next step is to list down all the possible models. The possible models for independent variables are shown in the Table 6.

Table 6. Labeling of all possible models with five independent variables

Number of Variables	Individual	INTERACTION				
		First Order	Second Order	Third Order	Fourth Order	TOTAL
1	5	-	-	-	-	**5**
2	10	10	-	-	-	**20**
3	10	10	10	-	-	**30**
4	5	5	5	5	-	**20**
5	1	1	1	1	1	**5**
TOTAL	**31**	**26**	**16**	**6**	**1**	**80**
Model Number	**M1-M31**	**M32-M57**	**M58-M73**	**M74-M79**	**M80**	

The next step is to estimate the coefficients for the entire possible model and carry out the test to get selected models. All the possible models are subjected to global test and coefficient test (based on p-value). For illustration purpose, consider model M67 where Table 8 shows the p-value for each variable of the model and Table 7 represents the ANOVA table for global test. The hypothesis of global test for model M67 as follows:

H_o: $\beta_3 = \beta_4 = \beta_5 = \beta_{34} = \beta_{35} = \beta_{45} = \beta_{345} = 0$
H_1: at least one β's is nonzero

As can be seen from Table 7, the F_{cal} is 11.3096 and F_{table} is $F_{0.05,7,55} = 2.21$. Since the F_{cal} is greater than F_{table} the decision is to reject the null hypothesis where all the regression coefficients are zero.

Table 7. The ANOVA of global test for model M67

Source of variations	Sum of Squares	df	Mean Square	F	p-value
Regression	47662.5930	7	6808.9419	11.3096	0.000
Residual	33112.8413	55	602.0517		
Total	80775.4343	62			

The next step is to search insignificant variable by performing the coefficient test for all the coefficients in the model. The hypothesis of coefficient test for β_3 is as follows:

H_o: $\beta_3 = 0$
H_1: $\beta_3 \neq 0$

The t_{cal} is -0.0691 and the t_{table} is $t_{0.025,55}$ = 2.01. The decision is to accept the null hypothesis where regression coefficient is zero since $|t_{cal}|$ is less than $|t_{table}|$. Similar procedure is carried out for other coefficients in the model where the test leads to the elimination of variable (highest p-value > 0.05).

Table 8. Procedure in getting a selected model (M67.3)

Variables	Models			
	M67	**M67.1**	**M67.2**	**M67.3**
Constant	-8.9338	1.6340	-5.7871	-7.3286
X_4	2.7108 (0.7364)	2.4353 (1.5699)	3.3083 (2.4595)	1.0189 (2.0667)
X_5	37.5128 (0.5368)	32.0733 (1.3723)	55.4886 (5.2592)	56.8921 (5.3013)
X_{45}	-2.4958 (-1.0596)	-2.3372 (-1.7241)	-3.3955 (-3.4794)	-1.8246 (-3.8994)
X_{345}	0.6012 (0.7919)	0.5489 (1.3206)	0.8632 (2.8050)	0.3233 (3.7706)
X_{34}	-0.6479 (-0.5320)	-0.5558 (-1.1372)	-0.8000 **(-1.8239)**	
X_{35}	4.4210 (0.1977)	6.2114 **(1.1221)**		
X_3	3.5329 **(0.0827)**			
SSE	33112.8413	33116.958	33861.597	35837.7441
***$t_{critical}$** = 2.01 and value in parentheses is the t_{cal}				

As can be seen from Table 8 (model M67), each variable has p-value higher than 0.05 which means that the corresponding independent variable is insignificant. Hence, by omitting the variable with highest p-value that is variable X_3 (p-value =0.934) and rerun the analysis with remaining variables. The resulting p-value after eliminating variable X_3 is shown in Table 8 (model M67.1). From Table 8, the variables in the new regression equation, M67.1 are not significant because all the variables had p-value larger than 0.05. The interaction variable X_{35} (p-value =0.267) had been omitted from the model and the analysis was rerun with the remaining variables. The new set of p-values after eliminating variable X_{35} is shown in Table 8 (model M67.2). As can be seen from Table 6 (M67.2), the interaction variable X_{34} is not significant (p-value > 0.05), X_{34} is omitted from the model and the analysis is rerun. The p-values after eliminating variable X_{34} are shown in Table 8 (model M67.3).

The Table 8 (last column, model M67.3) shows that all the remaining independent variables are significant where the p-value of each variable is less than 0.05. Thus, after the 3 variables had been omitted a selected model is obtained i.e. model M67.3 where $Y = -7.329 + 1.019X_4 + 56.892X_5 - 1.825X_{45} + 0.323X_{345}$. The following step is the Wald test. Wald Test is carried out to the final model where the restricted model (model M67.3) is the selected model and unrestricted model is the initial possible model (model M67).

The unrestricted model (Possible Model): M67

U: $Y = \beta_0 X_0 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_{34} X_{34} + \beta_{35} X_{35} + \beta_{45} X_{45} + \beta_{345} X_{345} + u$

SSE(U) = 33112.8413 and df(U) = 55

The restricted model (Selected Model): M67.3

R: $Y = \beta_0 X_0 + \beta_4 X_4 + \beta_5 X_5 + \beta_{45} X_{45} + \beta_{345} X_{345} + r$

SSE(R) = 35837.7441 and df(R) = 58

The hypothesis of Wald test for removing variable X_3, X_{34} and X_{35} from model M67 is as follows:

H_o: $\beta_3 = \beta_{34} = \beta_{35} = 0$

H_1: at least one β's is nonzero

Table 9. Wald test for model M67 and model M67.3

Source of variations	Sum of Squares	*df*	Mean Sum of Squares	F
Differences	2724.9028	3	908.3009	1.5087
Unrestricted (U)	33112.8413	55	602.0517	
Restricted (R)	35837.7441	58		

From Table 9, the F_{cal} is 1.5087 and F_{table} is $F_{0.05,3,55} = 2.8000$. The decision is to accept the null hypothesis where all the regression coefficients are zero since F_{cal} is less than F_{table}. Thus, this is justifying the removal of the insignificant variables in coefficient test. Similar procedures are carried out on all possible models systematically. At the end of the procedure, altogether there are 47 selected models obtained and their summary is shown in Appendix A. For each selected model, find the value of each model selection criterion mentioned in Table 4 and corresponding values are shown in Appendix B. Majority of the criteria shown in Appendix B indicates that model M73.15 is the best model. Result of the coefficient test of the model M73.15 is shown in Table 10 (all the t-values for significant variables in model M73.15 are greater than 2.01).

Table 10. Possible Model (M73) to Selected Model (M73.15)

Variables	Models					
	M73	**M73.1**	**M73.6**	**M73.10**	**M73.10**	**M73.10**
Constant	326.0692	151.0304	243.8263	163.4443	103.5039	101.8905
X_2	-55.7326 (-1.232)	-32.3452 (-0.618)	-53.3366 (-2.613)	-42.8108 (-3.002)	-31.8661 (-3.411)	-26.8289 (-3.5995)
X_4	-6.0270 (-1.825)	-3.2878 (-0.608)	-4.7949 (-1.636)	-3.6592 (-1.713)	-2.6870 (-3.635)	-2.6146 (-3.5647)
X_{12}	0.0661 (1.749)	0.0485 (1.764)	0.0547 (2.982)	0.0499 (3.283)	0.0412 (4.581)	0.0411 (4.5809)
X_{15}	-0.243 (-0.034)	-0.0679 (-0.641)	-0.0464 (-0.797)	-0.0564 (-1.763)	-0.0738 (-2.961)	-0.0744 (-2.9901)

X_{45}	4.2364 (1.570)	3.5972 (1.117)	3.6524 (1.637)	4.1793 (2.330)	3.5154 (3.248)	3.1277 (3.1579)
X_{123}	-0.0094 (-2.676)	-0.0127 (-2.058)	-0.0118 (-3.777)	-0.0115 (-4.065)	-0.0094 (-4.493)	-0.0091 (-4.4120)
X_{135}	0.0332 (1.306)	0.0373 (1.220)	0.0380 (3.047)	0.0344 (3.248)	0.0278 (3.659)	0.0277 (3.6539)
X_{145}	-0.0017 (-0.945)	-0.0020 (-1.019)	-0.0018 (0.969)	-0.0016 (-1.717)	-0.0012 (-2.819)	-0.0013 (-3.0432)
X_{234}	0.1691 (0.615)	0.1202 (0.431)	0.0983 (0.571)	0.1592 (3.372)	0.1628 (4.005)	0.1553 (3.9114)
X_{345}	-1.2196 (-1.129)	-0.8043 (-0.599)	-0.6873 (-0.721)	-0.7200 (-1.964)	-0.7418 (-2.248)	-0.5578 (-2.1618)
X_{23}	3.3030 (0.216)	9.8753 (0.697)	10.6384 (1.378)	6.2713 (1.427)	1.9904 **(0.897)**	-
X_{35}	-4.211 (-0.619)	-12.5710 (-0.189)	-16.7836 (-0.724)	-15.3869 (-1.040)	-	-
X_{1}	-0.1523 (-1.027)	-0.0529 (-0.283)	-0.0829 (0.730)	-0.0589 (-0.795)	-	-
X_{125}	-0.0110 (-0.773)	-4.295 (-0.480)	-0.0048 (-0.833)	-0.0017 (-0.488)	-	-
X_{14}	0.0024 (0.629)	0.0012 (0.274)	0.0014 (0.505	0.0006 **(0.431)**	-	-
X_{245}	0.2536 (0.761)	0.2407 (0.510)	0.1223 (0.344)	-	-	-
X_{3}	-14.0226 (-0.211)	-30.9192 (-0.398)	-30.6788 (-0.562)	-	-	-
X_{34}	0.6658 (0.411)	0.4884 (0.252)	0.6328 (0.446)	-	-	-
X_{134}	-2.964 (-0.308)	-4.419 (-0.393)	-0.0002 **(-0.249)**	-	-	-
X_{24}	-1.049 (-0.412)	-0.3726 (-0.294)	-	-	-	-
X_{25}	4.5162 (0.142)	-9.7373 (-0.604)	-	-	-	-
X_{5}	-82.6096 (-0.727)	37.7390 (0.201)	-	-	-	-
X_{13}	-0.0118 (-0.224)	0.0039 (0.118)	-	-	-	-
X_{124}	-0.0003 (-0.437)	0.000029 **(0.050)**	-	-	-	-
X_{235}	0.8859 **(0.137)**	-	-	-	-	-
SSE	14180.583	14231.763	14183.740	14376.6330	14839.247	15073.4451

***$t_{critical}$** = 2.01 and value in parentheses is the t_{cal}

Thus, the best model is M73.15 where

$Y=101.891–26.829X_2+0.41X_{12}–0.017X_{15}+3.128X_{45}–0.009X_{123}+0.028X_{135}–0.001X_{145}+0.155X_{234}–0.558X_{345}$

There are 15 variables omitted from the model M73. A Wald Test is carried out to the final model (Ramanathan, 2002) where the restricted model (model M73.15) is the selected model and unrestricted model is the initial possible model (model M73). The hypothesis of Wald test for removing variables X_1, X_3, X_5, X_{13}, X_{14}, X_{23}, X_{24}, X_{25}, X_{34}, X_{35}, X_{124}, X_{125}, X_{134}, $X_{235}$and X_{245} from model M73:

H_0: $\beta_1=\beta_3=\beta_5=\beta_{13}=\beta_{14}=\beta_{23}=\beta_{24}=\beta_{25}=\beta_{34}=\beta_{35}=\beta_{124}=\beta_{125}=\beta_{134}=\beta_{235}=\beta_{245}=0$

H_1: At least one βs is nonzero

Table 10. Wald test for model M73 and model M73.15

Source of variations	Sum of Squares	*df*	Mean Sum of Squares	F
Differences	892.8621	11	81.1693	0.2347
Unrestricted (U)	14180.5830	41	345.8679	
Restricted (R)	15073.4451	52		

Seven criteria had chosen model M73.15 and one criterion for model M75.11. F_{table} =F(11, 41, 5%) =2.03. Since F_{calc} is less than F_{table}, H_0 is accepted. Thus, this is justified. The similar procedure of Wald Test is carried out for all other selected models and same results are obtained. Based on the best model, the predicted Y was determined. Using the residuals obtained, randomness test is carried out. Both randomness test and residual scatter plot indicate that the residuals are random and independent. That means, the model M73.15 is the best model to describe the house selling price in Ohio and it's ready to be used for further analysis.

DISCUSSION AND CONCLUSION

Since the effect of higher order interaction existed significantly, polynomial of higher order interaction should be included in the possible models. Other variables such as number of garage, location of the house and other relevant characteristics should be considered for future work. The work shows that model M73.15 is the best model to describe the house selling price in Ohio.

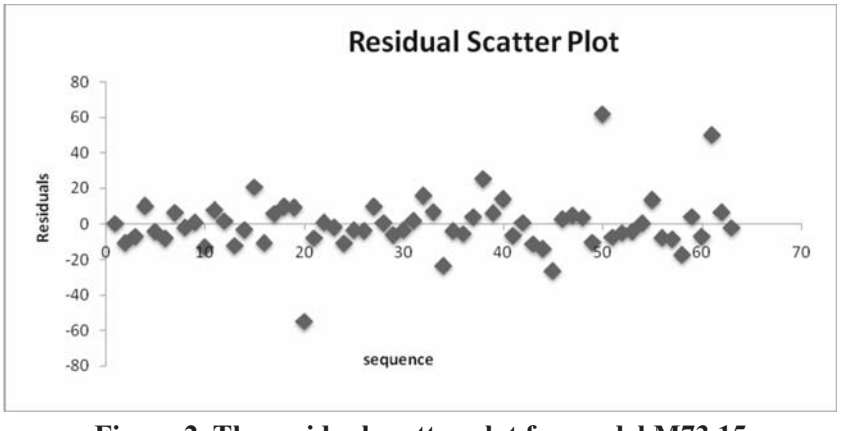


Figure 2. The residual scatter plot for model M73.15

Now the house selling price model is ready for forecasting to make a logical decision to determine the house selling price. The total sum of residual of the best model (model M73.15) is 4.9396×10^{-9} (very small and almost near to zero) while the mean sum of square error is 289.874.

The randomness test carried out on residuals shows that resulting error term of model M73.15 is random and independent. This is reflected in the residuals scatter plot of Figure 2 which shows no obvious pattern existed. Model M73.15 also shows that there exists significant interaction effect. The floor area and number of rooms interact together. The Figure 3 shows the the error terms are normally distributed since the residuals form a bell shape.

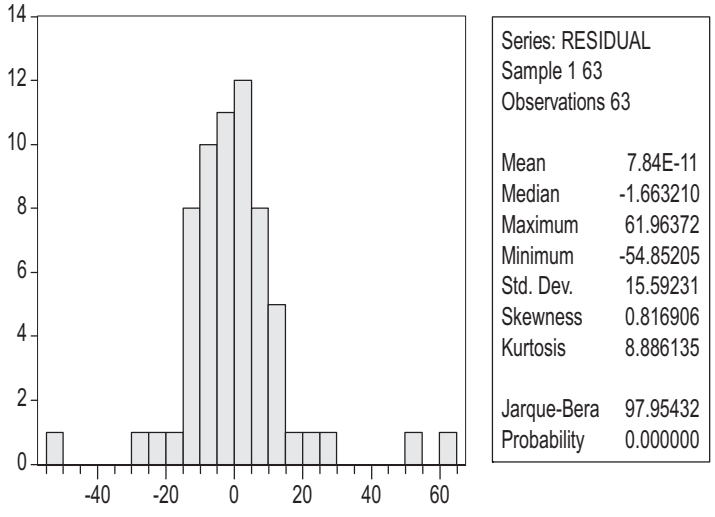


Figure 3. The descriptive statistics and histogram of the residual from model M73.15

This model shows that the variables: the floor area, number of bedrooms and number of bathrooms, do not have direct effect on this selling price of a house. These variables cannot act as single effect variable. The number of rooms and age can have direct effect in determining the house selling price. But when number of rooms or age increase, the house selling price decreases. This model also shows that, to determine a house selling price, the variables should interact with each other. Based on the best model, it can be concluded that in order to determine a house selling price, one should consider house characteristics like floor area, number of rooms, number of bedrooms, age of the house and number of bathrooms. Besides these variables, a person's willingness/readiness to buy a house, income status, and basic facilities around the housing areas can also affect the house selling price.

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APPENDIX A: THE SELECTED MODELS

Model	The Selected Models	k+1	SSE
M1	M1	**2**	30998.9710
M2	M2	**2**	53609.0350
M3	M3	**2**	59601.8130
M4	M4	**2**	74012.6580
M5	M5	**2**	46527.2500
M8	M8	**3**	27603.1960
M9	M9	**3**	27654.2200
M11	M11	**3**	41096.4610
M12	M12	**3**	36779.0590
M13	M13	**3**	52416.2600
M14	M14	**3**	39148.0960
M24	M24	**4**	34185.3180
M34	M34.1	**3**	27328.9650
M35	M35.1 **=>** M35.2	**2**	28355.1480
M36	M36.1 **=>** M36.2	**2**	52105.2640
M37	M37.1	**3**	40397.9520
M38	M38.1 **=>** M38.2	**2**	35395.2180
M39	M39.1	**3**	51824.6620
M40	M40.1 => M40.2	**2**	36751.5540
M43	M43.1 => M43.2	**4**	24045.0190
M44	M44.1 => M44.2 => M44.3 => M44.4	**3**	27524.7090
M46	M46.1 => M46.2 => M46.3	**6**	23804.6840
M48	M48.1 => M48.2 => M48.3 => M48.4	**3**	39493.5960
M50	M50.1 => M50.2 => M50.3 => M50.4	**3**	31466.8550
M52	M52.1 => M52.2 => M52.3 => M52.4 => M52.5 => M52.6	**5**	22116.7010
M53	M53.1 => M53.2 => M53.3 => M53.4 => M53.5 => M53.6 => M53.7	**4**	26426.4150
M54	M54.1 => M54.2 => M54.3 => M54.4 => M54.5 => M54.6 => M54.7	**4**	24110.5790
M57	M57.1 =>… => M57.10	**6**	21774.7130
M58	M58.1 => M58.2 => M58.3 => M58.4	**4**	26735.4780
M59	M59.1 => M59.2 => M59.3 => M59.4	**4**	25591.7630
M60	M60.1 => M60.2 => M60.3	**5**	24703.7830
M62	M62.1 => M62.2 => M62.3 => M62.4	**4**	25499.6560
M63	M63.1 => M63.2 => M63.3 => M63.4	**4**	25837.9670
M66	M66.1 => M66.2 => M66.3 => M66.4 => M66.5	**3**	32497.3660
M67	M67.1 => M67.2 => M67.3	**5**	35837.7440
M68	M68.1 =>… => M68.8	**7**	19300.7880
M69	M69.1 =>… => M69.9	**6**	21734.8560
M70	M70.1 =>… => M70.10	**5**	22732.4740
M71	M71.1 =>… => M71.10	**5**	24178.5540
M72	M72.1 =>… => M72.11	**4**	29244.9580
M73	M73.1 =>… => M73.15	**11**	15073.4450
M74	M74.1 =>… => M74.10	**7**	19300.7880
M75	M75.1 =>… => M75.11	**5**	21565.1040
M76	M76.1 =>… => M76.9	**7**	20962.8220
M77	M77.1 =>… => M77.12	**4**	25499.6560
M79	M79.1 =>… => M79.22	**9**	16634.4070
M80	M80.1 =>… => M80.19 (this model has the minimum SSE)	**13**	14840.3610

APPENDIX B: THE EIGHT MODEL SELECTION CRITERIA VALUES FOR THE CORRESPONDING SELECTED MODELS

Selected Model	SELECTION CRITERIA							
	AIC	FPE	GCV	HQ	RICE	SCHWARZ	SHIBATA	SGMASQ
M1	524.301	524.313	524.841	538.520	525.406	561.214	523.288	508.180
M2	906.717	906.736	907.651	931.307	908.628	970.553	904.965	878.837
M3	1008.076	1008.097	1009.114	1035.415	1010.200	1079.048	1006.128	977.079
M4	1251.814	1251.840	1253.103	1285.763	1254.452	1339.946	1249.395	1213.322
M5	786.939	786.956	787.750	808.281	788.597	842.343	785.418	762.742
M8	481.926	481.961	483.056	501.663	484.267	533.706	479.874	460.053
M9	482.817	482.851	483.949	502.590	485.162	534.692	480.761	460.904
M11	717.506	717.557	719.188	746.891	720.991	794.597	714.451	684.941
M12	642.128	642.174	643.634	668.426	645.247	711.120	639.394	612.984
M13	915.139	915.205	917.285	952.618	919.584	1013.464	911.243	873.604
M14	683.489	683.538	685.092	711.481	686.809	756.925	680.579	652.468
M24	616.095	616.200	618.694	649.965	621.551	705.900	611.529	579.412
M34.1	477.138	477.172	478.257	496.679	479.456	528.403	475.107	455.483
M35.2	479.585	479.595	480.079	492.591	480.596	513.350	478.658	464.838
M36.2	881.283	881.302	882.191	905.183	883.140	943.329	879.580	854.185
M37.1	705.310	705.361	706.964	734.196	708.736	781.091	702.308	673.299
M38.2	598.657	598.670	599.274	614.893	599.919	640.805	597.501	580.249
M39.1	904.810	904.875	906.932	941.866	909.205	1002.026	900.958	863.744
M40.2	621.598	621.611	622.238	638.456	622.908	665.361	620.397	602.484
M43.2	433.344	433.418	435.173	457.168	437.182	496.510	430.133	407.543
M44.4	480.556	480.590	481.682	500.237	482.890	532.188	478.510	458.745
M46.3	457.135	457.400	461.587	495.346	466.759	560.645	449.824	417.626
M48.4	689.521	689.571	691.138	717.760	692.870	763.605	686.586	658.227
M50.4	549.382	549.421	550.670	571.881	552.050	608.409	547.043	524.448
M52.6	411.448	411.586	414.195	439.915	417.296	487.736	406.782	381.322
M53.7	476.262	476.344	478.272	502.445	480.480	545.684	472.733	447.905
M54.7	434.526	434.600	436.359	458.414	438.374	497.864	431.305	408.654
M57.10	418.152	418.395	422.224	453.105	426.955	512.835	411.465	382.013
M58.4	481.832	481.915	483.865	508.321	486.100	552.066	478.261	453.144
M59.4	461.220	461.299	463.166	486.576	465.305	528.450	457.802	433.759
M60.3	459.577	459.731	462.645	491.373	466.109	544.788	454.365	425.927
M62.4	459.560	459.639	461.499	484.825	463.630	526.548	456.154	432.198
M63.4	465.657	465.737	467.622	491.257	469.781	533.533	462.206	437.932
M66.5	567.373	567.414	568.704	590.610	570.129	628.334	564.958	541.623
M67.3	666.708	666.931	671.159	712.835	676.184	790.324	659.147	617.892
M68.8	382.599	382.952	387.739	420.164	393.894	485.469	374.442	344.657
M69.9	417.387	417.629	421.452	452.275	426.174	511.896	410.712	381.313
M70.10	422.904	423.045	425.727	452.163	428.915	501.315	418.108	391.939
M71.10	449.806	449.957	452.809	480.926	456.199	533.205	444.705	416.872
M72.11	527.059	527.149	529.282	556.034	531.727	603.885	523.152	495.677
M73.15	**339.258**	**340.487**	**351.193**	**393.049**	**367.645**	493.222	**322.813**	**289.874**
M74.10	382.599	382.952	387.739	420.164	393.894	485.469	374.442	344.657
M75.11	401.187	401.321	403.865	428.943	406.889	**475.571**	396.637	371.812
M76.9	415.546	415.929	421.128	456.345	427.813	527.273	406.686	374.336
M77.12	459.560	459.639	461.499	484.825	463.630	526.548	456.154	432.198
M79.22	351.359	352.051	359.385	396.320	369.653	477.216	339.478	308.045
M80.19	355.907	358.053	373.977	423.521	401.091	553.855	332.777	296.807

The highlighted values are the minimum value for each criterion. Thus, model M73.15 is chosen the best model due to the fact that seven of the eight criteria having the least value.

PENENTUAN KOMPONEN MERUAP DALAM TIGA BENTUK BERBEZA GULA MELAKA DENGAN MENGGUNAKAN KAEDAH MIKROEKSTRAKSI (FASA) PEPEJAL

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ABSTRAK. *K*ajian ini dijalankan untuk menentukan perbezaan komponen meruap yang terdapat dalam gula melaka yang berbeza bentuk iaitu serbuk, bulat dan silinder yang terdapat di pasaran. Kaedah yang digunakan adalah *headspace* mikroekstraksi fasa pepejal yang digandingkan dengan kromatografi gas spektrometri jisim. Masa pemanasan minit dan suhu pemanasan 60°C digunakan untuk menentukan kepekatan komponen meruap. Hasil daripada GC/MS menunjukkan sebanyak 24 komponen meruap hadir dalam gula melaka serbuk, 26 komponen bagi gula melaka berbentuk bulat dan 22 komponen hadir dalam bentuk silinder. Ada lebih daripada 90 komponen meruap yang hadir bagi kesemua bentuk sampel gula melaka. Bagi gula melaka silinder komponen major adalah daripada kumpulan furan iaitu 2-furankarboksaldehid (32.27%) dan 2-furanmetanol (9.78%). Bagi gula melaka berbentuk bulat komponen majornya ialah nonanal (13.67%) dan oktanal (7.55%) manakala gula melaka serbuk komponen utamanya ialah trans-anetol (5.21%) dan 1-(1H-pyrol 2il)-etanon (4.21%) yang dikelaskan dalam kumpulan lakton. Analisis daripada sampel menunjukkan gula melaka berbentuk silinder mempunyai peratus komponen meruap yang paling tinggi diikuti gula melaka bulat manakala gula melaka serbuk mempunyai peratus komponen meruap yang paling rendah.

KATA KUNCI. Gula melaka, SPME, komponen meruap

ABSTRACT. *This study was conducted to determine the difference in volatile compound between different shapes of palm sugar; powder, round and cylinder. The method used were headspace solid phase micro extraction and chromatography gas mass spectrometry (HS-SPME-GC/MS). Heating time was 30 minutes and heating temperature 60°C was used to determine the concentration of the volatile compound. GC/MS result showed that 24 volatile compounds were present in powder shape, 26 compounds in round shape and 22 compounds in cylinder shape. There were more than 90 volatile compounds found in each of the sample shape. For cylinder palm sugar, major compound identified was from furan group namely 2-furancarboksaldehyde (32.27%) and 2-furanmethanol (9.78%). For round palm sugar, nonanal (13.67%) and octanal (7.55%) were the major compounds and as for powder palm sugar, the compound presented were trans-anetol (5.21%) and 1-(1H-pyrol 2il)-etanon (4.21%) which were from phenol and lactone group respectively. Analysis from the samples showed that cylinder shape contained the highest percentage of volatile compounds, followed by round shape palm sugar whereas powder palm sugar has the lowest percentage of volatile compounds.*

KEYWORDS. Palm sugar, SPME, volatile compound

PENGENALAN

Gula melaka atau dikenali juga dengan nama gula tuak banyak terdapat di negara asia. Ia juga dikenali dengan pelbagai nama seperti Nam Taan Oi di Thailand, Gur di India, Jaggery di Burma dan Sri Lanka serta gula Jawa di Indonesia. Ia biasanya berbentuk silinder, pepejal bulat dengan ketebalan berbeza ataupun dalam bentuk serbuk. Warna gula melaka biasanya bewarna coklat gelap atau terang. Warnanya juga berbeza dengan gula perang atau gula merah yang diproses daripada tebu.

Gula melaka merupakan salah satu daripada sumber manisan di Malaysia. Gula ini diperbuat daripada nira kelapa dan digunakan dalam makanan sebagai perisa untuk mendapatkan rasa berlemak, karamel dan untuk bau yang harum dalam masakan. Kasma (1995) menyatakan gula melaka adalah pemanis yang paling baik dan merupakan agen pengimbang dan penambah perisa dalam makanan.

Banyak teknik telah dijalankan untuk diaplikasi dalam proses pengekstrakan dan pemekatan kompone perisa saripada produk makanan. Sebilangan daripada teknik ini ialah teknik pengekstrakan pelarut, pengekstrakan soxhlet, persampelan ruang tutuan secara statik, persampelan ruang tutupan secara dinamik dan mikroekstraksi fasa pepejal (SPME). Dalam kajian ini, SPME digunakan kerana ia lebih stabil dan mampu mengumpul komponen meruap bagi sesuatu sampel. Melalui kaedah ini tiada tindakbalas di antara sampel dan bahan larutan berlaku kerana sampel dipanaskan secara langsung dan componen meruapnya terus dikumpul dan kemudian dianalisis dengan menggunakan gas kromatografi.

Komponen meruap yang tinggi tidak semestinya memberikan sifat aroma tertentu sesuatu produk. Komponen ini mungkin memiliki nilai ambang yang rendah dalam makanan tersebut maka ia akan memberikan bacaan nilai aroma yang tinggi. Komponen perisa yang telah dipisahkan daripada produk makanan perlu dipekatkan dan dianalisis untuk mengenalpasti setiap komponen yang hadir dan kemudian komponen ini akan ditentukan kepekatanya dan komposisi mana yang memberikan nilai secara signifikan kepada perisa makanan ini. Oleh it, analisis dengan menggunakan kromatografi gas spektrometer jisim akan perlu digunakan bersama-sama dengan mikroekstraksi fasa pepejal.

Menurut Apriyantono (2002), komposisi komponen meruap gula melaka sering didominasi oleh produk hasil daripada tindakbalas pemerangan seperti furan dan pirazin. Selain itu, produk daripada degradasi lipid seperti asid lemak dan keton juga akan menyumbang kepada profil komponen meruap yang dihasilkan daripada gula melaka. Komponen aroma meruap juga terdiri daripad asid organik iaitu asid sitrk, suksinik dan laktik.

Terdapat banyak faktor yang menyumbang kepada aroma yang dihasilkan oleh gula melaka. Menurut Siti Hasidah & Suchi Sihartini (1988), komponen yang menghasilkan aroma ini dihasilkan daripada cara penyediaan gula melaka itu sendiri dan juga tapak jalan metabolik yang terlibat di dalam air nira. Terdapat banyak kajian yang telah dijalankan dalam menentukan komponen aroma yang dihasilkan daripada tindakbalas Maillard dan juga kajian mengenai tahap pemerangan semasa penyediaan gula melaka (Shaaban & Samsudin, 1989). Ada juga kajian dijalankan mengenai air nira kelapa itu sendiri (Apriyantono et.al., 2002) tetapi hanya sedikit sahaja kajian yang telah dijalankan untuk menentukan dan mengelaskan aroma yang terhasil selepas tindakbalas Maillard dalam gula melaka. Oleh itu kajian ini dijalankan untuk menentukan komponen meruap yang terhasil semasa pemanasan gula melaka yang berlainan bentuk dengan menggunakan masa dan suhu yang ditetapkan.

BAHAN DAN KAEDAH

Bahan

Gula melaka daripada nira air kelapa dan diproses menjadi bentuk serbuk, silinder dan bulat tanpa jenama dibeli di kedai yang terdapat di Melaka.

Penyediaan Sampel

Penyediaan sampel dilakukan di dalam bilik sejuk bersuhu 4°C untuk mengelakkan kehilangan komponen meruap. Gula melada yang berbentuk silinder dan bulat dipotong kepada kepingan kacil berukuran kira-kira 2cm panjang dan 2cm lebar. Setelah kesemua gula melada dipotong kecil, 50 gram gula melaka kemudiannya dikisar selama 30 saat dengan menggunakan mesin pengisar jenis Khind model BL 310N bervolatan 220-240V.

Pengekstrakan Komponen Meruap

Pengekstrakan komponen meruap dilakukan dengan menggunakan kaedah mikro ekstraksi fasa pepejal (SPME). Sebanyak satu gram sampel gula melaka dimasukkan ke dalam vial yang berpenutup septum. Sampel dipanaskan dengan menggunakan blok pemanas bersuhu 60°C. Jarum SPME dicucuk masuk ke dalam septum vial dan fiber jenis 75µm polidimetilsiloksan (PDMS) didedahkan kepada sampel selama 30 minit untuk mendedahkan fiber kepada sampel. Kemudian fiber akan dipindahkan kepada bilik suntikan GC dan analisis kromatografi dijalankan. Setiap analisis dilakukan secara triplikat.

Analisis Kromatografi Spektrometri Jisim (GC/MS)

Penentuan profil sampel dijalankan dengan menggunakan model gas kromatografi *Hewlett Packard 6890 series GC system* dan model spektrometer adalah *Hewlett Packard 5972 series Mass Selective Detector.* Kolum kapilari supelcowex tidak polar (ketebalan filem 0.25µm) digunakan bersama helium bertekanan 2.0 psi. Sampel gula melaka diseimbangkan dalam *headspace* selama 20 minit pada 50°C selepas disuntik. Program kolum suhu awal oven adalah 50°C selama 5 minit kemudian dinaikkan sebanyak 20°C /min sehingga mencapai suhu 230°C dengan suhu pengesan setinggi 280°C.

HASIL DAN PERBINCANGAN

Profil Komponen Meruap Gula Melaka

Jadual 1 menunjukkan komponen meruap yang hadir bagi sampel gula melaka yang mempunyai bentuk yang berbeza.

Jadual 1. Komponen meruap bagi sampel gula melaka pada suhu 60°C

Komponen	Masa Penahanan	% Keluasan Puncak		
		Silinder	Bulat	Serbuk
Heksanal	2.71	0.37	1.74	0.36
Heptanal	3.92	-	2.12	-
Oktanal	5.05	1.64	7.55	1.28
Benzaldehid	5.54	-	1.84	1.59
Nonanal	6.16	3.23	13.67	3.57
Dekanal	7.32	0.49	1.98	0.64
2-Decenal	7.98	1.43	-	-
Undekanal	8.54	-	1.41	0.27
2-dodecenal	9.24	0.41	1.44	-
n-dodekanal	9.79	0.71	1.78	0.29
Feniletil Alcohol	2.52	-	4.54	-
2-etil heksanol	5.33	-	2.29	-
n-oktanol	5.77	0.73	3.78	-
Etanol	4.81	2.27	-	1.32
2,2-dimetil-4-metoksi-3(2H)-furanone	2.80	0.46	0.78	0.42
2-furankarboksaldehid	3.12	32.27	1.39	-
2-furanmetanol	3.38	9.78	3.81	1.85
2-furankarboksaldehid 5-metil	4.77	4.72	-	-
2-furankarboksaldehid 5-hidroksim	7.58	1.01	-	-
Acetamida	1.61	1.71	3.10	-
1-(2-furanil)-etanon	4.06	11.76	-	-
1-(1H-pirol 2 il)-etanon	5.71	-	1.88	4.21
3-hidroksi-2-metil-4H-piran-4-on	6.63	1.85	-	2.34

Setiap komponen yang hadir dalam gula melaka adalah berbeza-beza mengikut bentuk gula melaka. Bagi gula melaka berbentuk bulat, komponen aldehid adalah paling banyak hadir iaitu sebanyak 9 komponen berbanding silinder dan serbuk iaitu tujuh komponen masing-masing. Peratus jumlah keluasan puncak bagi kumpulan aldehid bagi gula melaka silinder, bulat dan serbuk ialah 8.3%, 33.52% dan 7.98% masing-masing. Masa yang diperlukan untuk menahan komponen meruap daripada kumpulan aldehid ini adalah daripada minit pemanasan ke 2.71 sehingga minit ke 9.79.

Komponen meruap yang paling tinggi dalam kumpulan aldehid adalah nonanal bagi gula melaka bulat dan serbuk manakala komponen oktanal bagi gula melaka silinder. Menurut Burdock (2001), nonanal memberikan bau berlemak dan bunga mawar manakala oktanal menyumbang kepada bau yang tajam pada gula melaka.

Selain daripada kumpulan aldehid, kumpulan alkohol turut hadir dalam kesemua sampel tersebut. Komponen meruap yang dikelaskan dalam kumpulan ini ditahan pada masa penahanan ke 2.52 sehingga minit ke 4.81. Fenil etil alcohol (4.54%) mempunyai kepekatan paling tinggi manakala manakala n-oktanal (1.32%) adalah paling rendah bagi kesemua sampel. Alkohol secara relatifnya hanya memberikan bau yang lemah dan ia merupakan komponen permulaan untuk menghasilkan aldehid dan ester. Alcohol juga boleh menghasilkan bau seperti tumbuhan hijau dan bau bunga segar (Burdock, 2001).

Komponen yang dikelaskan dalam kumpulan furan juga hadir dalam sampel gula melaka. Walaubagaimanapun, hanya komponen 2,5-dimetil-4-metoksi-3(2H)-furanone (32.27%) dan 2-furanmetanol hadir dalam kesemua sampel gula melaka. 2-furanmetanol (9.78%) mempunyai keluasan puncak yang paling tinggi dalam gula melaka bentuk silinder. Dalam tindakbalas Maillard, furan akan terhasil jika ketosa digunakan dalam fasa penyusunan semula amadori. Penggunaan ketosa akan menghasilkan ketosilamina di mana komponen ini terlibat dalam penyusunan semula Heyns untuk menghasilkan 2-amino-2-dioksialdosa. Komponen ini bertindak sebagai prekursor untuk menghasilkan perisa yang mana akan menghasilkan furan dan terbitannya Fisher & Scott, 1997).

Furan akan menghasilkan ciri-ciri aroma seperti karamel, bau manis dan aroma seperti kacang. 2,5-dimetil-4-metoksi-3(2H)-furanone adalah kumpulan furan yang berstruktur kiral dan memberikan komponen aroma yang kuat. Ia memberikan aroma dalam kebanyakan buah-buahan dan juga dalam makanan terproses. Manakala bagi 2-furanmetanol, komponen ini menyumbang lepada rasa hangit, manis dan karamel kepada gula melaka (Curioni & Bosset, 2002).

Berdasarkan kepada jadual 1, didapati tidak semua komponen daripada kumpulan lakton hadir dalam ketiga-tiga sampel gula melaka. komponen 1-2-furanil)-etanon (11.76%) memberikan nilai peratusan puncak yang paling tinggi dalam gula melaka silinder manakala bagi gula melaka bulat komponen meruap kumpulan lakton yang mempunyai peratusan paling tinggi ialah asetamida (3.10%) dan bagi gula melaka serbuk ialah 1-(1H-pirol 2-il)-etanon (4.21%). Lakton biasanya dikaitkan dengan aroma buah pic, aprikot dan juga buah kelapa. Selain itu, ia juga menyumbang kepada banyak aroma buah-buahan dan mentega (Bauer, 1985).

Keton lazimnya dikenali dengan bau primernya seperti *surface mould ripened*. Bau ini dikaitkan dengan aktiviti enzimatik kulat. Dalam gula melaka berbentuk silinder dan serbuk, komponen 3-metil-2-siklopenten-1-one iaitu komponen yang dikelaskan dalam kumpulan keton dikenalpasti sebagai salah satu komponen yang menyumbangkan perisa karamel dalam gula melaka.

Jadual 2. Asid meruap yang hadir pada suhu 60°C bagi gula melaka berlainan bentuk

Asid	% Kawasan Keluasan Puncak		
	Silinder	Bulat	Serbuk
Asid asetik	12.89	12.29	40.24
Asid isovalerik	-	1.45	2.68
Asid oktanoik	3.49	1.36	5.10
Asid heksadekanoik	0.95	8.67	4.11

Berdasarkan jadual 2 didapati asid juga hadir dalam peratusan keluasan puncak yang tinggi semasa pemerangkapan komponen meruap. Kebanyakan asid ini terhasil melalui lipólisis trigliserida, degradasi asid amino dan tindak balas pengoksidaan (Curioni & Bosset, 2002).

KESIMPULAN

Berdasarkan kepada kromatografi yang dijalankan, diperhatikan bahawa tidak semua komponen yang hadir dalam gula melaka serbuk juga akan hadir dalam gula melaka berbentuk bulat dan silinder dan sebaliknya. Didapati gula melaka berbentuk bulat mempunyai komponen aroma meruap yang lebih banyak diikuti dengan gula melaka berbentuk serbuk dan silinder. Walaubagaimanapun, kuantiti aroma meruap tidak semestinya akan mencirikan sesuatu gula melaka tersebut.

Kebanyakan aroma yang terkandung dalam gula melaka terhasil daripada tindakbalas Maillard dan sebahagian lagi wujud secara semulajadi dalam air nira kelapa. Aroma ini juga terhasil daripada pemanasan dan pengkaramelan iaitu semasa proses pemasakan air nira sebelum membentuk gula melaka. Setiap komponen yang hadir menyumbang dalam menghasilkan perisa yang tersendiri kepada gula melaka.

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WATER ABSORPTION AND CURING TIME PERFORMANCE OF UREA FORMALDEHYDE RESIN MIXED WITH DIFFERENT AMOUNT OF PHOSPHOROUS-BASED FIRE RETARDANTS

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ABSTRACT. *The curing time and the properties of urea formaldehyde (UF) resin mixed with fire retardants, BP (mixture of boric acid, guanylurea phosphate and phosphoric acid), monoammonium phosphate (MAP) and diammonium phosphate (DAP) were studied. There were two amounts used, 8% w/w and 10% w/w. The curing time of the mixed resin was determined by using thermo oil at the temperature of 170ºC. Water absorption test and physical observations were done to evaluate the properties of the fire retardant-mixed resin. The non-fire retardant UF resin samples were used as controls. The solubility of MAP and DAP in the water at different weights was also studied. The solubility test was done with and without the involvement of heat. The study showed that UF resin mixed with MAP and BP cured faster than DAP-mixed UF and control samples. The time taken for UF resin to mix with 10 % w/w and 8 % w/w MAP were 20 s and 28 s respectively. The time taken for UF resin mixed with 10 % and 8 % w/w DAP were 160 s and 150 s respectively. The time taken for UF resin mixed with 10 % w/w and 8 % w/w BP were 101 s and 92 s respectively.The curing time for control samples was 140 s respectively. MAP and DAP were shown to be highly soluble, as they took less than 1 minute to be dissolved in the water without heat, but BP took 30 minutes to be dissolved in the water without heat and less than 1 minute with heat. Water absorption test showed that the higher the amount of MAP, DAP and BP mixed into the resin, the higher would be the rate of water absorbed.*

KEYWORDS. Monoammonium phosphate, diammonium phosphate, boron-phosphorous-based, solubility.

INTRODUCTION

Fire retardants used in this research are BP® (mixture of 27-33 % boric acid, 67-73 % guanylurea phosphate and 0.0-4.2 % phosphoric acid), monoammonium phosphate (MAP) and DAP (diammonium phosphate). MAP and DAP are two of the most popular fire retardants which usually used in producing fire retardant-treated panels. Besides of being used as fire retardants, DAP is alkaline (pH 7.2-8.0) and MAP is acidic (pH 3.5-4.5) in nature and usually used as fertilisers to increase or reduce the pH of soils (Anon, 2009a;Anon,2009b). BP® is one of the latest fire retardants in the world (Anon, 1991). BP is known as a fire retardant with high molecular, very limited solubility and has a good stability in higher temperature (Anon, 1991). Based on previous research done by (Izran *et al.*, 2008), pH of BP is weak acid with pH 6. In producing panel products, the alkalinity and acidity of those fire retardants may cause problems, especially when the resins used are pH sensitive.

The use of urea formaldehyde resin as a major adhesive by the forest products is due to many advantages it has. The advantages are low cost, ease of use under a wide variety of curing conditions, low cure temperature, water solubility, and resistance to microorganisms and to abrasion, hardness, and excellent thermal properties (Anthony, 1999). However, DAP, MAP and BP may affect the curing time of the resin in manufacturing particleboard as UF resin is sensitive to either alkalinity or acidity (Zaidon *et al.*, 2004). Gelation time of the resin used for particleboard fabrication seemed very important to help researchers to calculate sufficient hot pressing time for particleboard that will be fabricated. The method of observing the gelation time was implemented by Zaidon *et al.* (2004), Zaidon *et al.* (2007) and Zaidon *et al.* (2008). Calculation of sufficient hot pressing period through curing time is essential as insufficient hot pressing may cause adverse effects to the panels produced. It has been found that the increment of pH of urea formaldehyde, obviously affected the shear strength of wood. He also found that the glueline failure of UF increased with pH value (Freemen, 1959). In this study, the gelation time method was used to observe the effects of the phosphorous-based fire retardants to the gelation time of UF resin. It is expected that the study can provide useful information which can helps other researchers to do further research in order to overcome adverse effects that may happen when mixing urea formaldehyde resin with MAP, DAP and BP®.

MATERIALS AND METHODS

Curing time test

The curing test was done in order to determine the curing time of urea formaldehyde as it mix with fire retardants. This test is essential as it helps the researcher to calculate sufficient pressing time for panel product such as particle board mixed with fire retardant during hot pressing process. This method was implemented from Zaidon *et al.* (2004), Zaidon *et al.* (2007) and Zaidon *et al.* (2008).

BP®, MAP and DAP were obtained from Fire Protection Laboratory, Forest Research Institute Malaysia and EuroScience Sdn Bhd. Urea formaldehyde resin was obtained from Malayan Adhesive Chemicals Sdn. Bhd. The mixtures of fire retardants and UF resin later will be used as a binder to produce fire retardant–treated particle board with density 700 kg/m^3. Based on the calculation made, 193 g of UF resin, 29 g hardener and 17 g are required to produce the 700 kg/m^3 particle board. The ingredients (urea formaldehyde, hardener (ammonium chloride) and wax were then mixed and divided into two portions, one portion was mixed with 10 % w/w fire retardant and another portion was mixed with 8 % w/w fire retardant. The mixture was stirred using glass rod until they mixed well and the pH of each mixture was determined using Whatman Full Range PH Determination Paper. 10 ml of the mixtures was poured into the test tubes and at the same time, 600 ml thermo oil was heated in a beaker until it reached 170ºC. The temperature of the thermo oil was measured by soaking the tip of the Temperature Detector into the thermo oil.

Once the temperature of the resin reached 170ºC, the test tube filled with the mixture was soaked into the hot thermo oil and the time taken for the mixture to cure was recorded. After the mixture has cured, the mixture was taken out from the thermo oil and was left for 5 minutes at ambient temperature, so that the cured mixture can easily be taken out from the test tube. Before the properties of the mixture were observed, it was labelled. Then, the cured mixture was weighed to obtain the green weight and were kept in the oven at $^+$-105º C for a day / until it achieves constant oven-dry weight. The data of green and oven-dry weight is essential to know the moisture content of the samples. Ten replicates were produced for the test.

Solubility Test

A solubility test has been conducted to study the solubility of the fire retardants in the water with the involvement of heat or otherwise. The information on the solubility of the fire retardants will be really helpful to the readers, who have intention to use the chemical in liquid form. According to Anon (2009a), MAP and DAP are high soluble fire retardants with solubility 90% to 100 %. However, for BP®, the previous research revealed that BP® has a very limited solubility (Anon, 1991). The method was implemented and modified from Anon (2007)

Solubility with and without heat

Two hundred ml of water was heated in a beaker until it reaches 100ºC. The temperature was determined using Temperature Detector. Then, 20cm^3 of fire retardant (to establish 10% concentration solution) and 18 cm^3 (to establish 8% concentration solution) were poured into the hot water and stirred using glass rod. The time taken for the fire retardant to achieve ultimate solubility was determined by using stopwatch and the pH of the solution was obtained using Whatman Full Range PH Determination Paper. The same procedure was used as above for solubility test without heat, but the water was not heated before the fire retardant was poured in. The temperature of the water was 27ºC – 30 ºC.

Water Absorption Test

Water absorption test also was done to the cured fire retardant-mixed UF resin to determine the hygroscopicity of the mixture when exposed to the water. At the same time, the results of water absorption test will be used to evaluate the effect of chosen fire retardants to the strength of the cured resin. The samples from each mixture were placed into the oven at a temperature of ±105ºC for 24 hours or as they achieved constant oven dry weight. The samples used were the same samples produced from curing time test. The samples then were put into the dessicator for 30 minutes and re-weighed for oven dry weight. After that, the re-weighed samples were left soaked in a beaker of water for 24 hours. On the next day, the samples were taken out from the water; dabbed dry and re-weighed once again to get wet weight.

The percentage of water absorbed (WA) was calculated by using Eq. 1.1 (Anon, 2009):

$$WA(\%) = WW - OW / OW \times 100\ \% \qquad (1.1)$$

Where,

WA : Water absorbed (%)
WW : Wet weight (g)
OW : Oven dry weight (g)

RESULTS AND DISCUSSION

Curing Time Test

The average time taken for 8% w/w MAP-mixed resin and DAP-mixed resin to cure was 28 seconds and 92 seconds respectively. The pH of MAP-mixed resin was 5 and DAP-mixed resin was 8. The average time taken for 10 % w/w MAP- mixed resin, BP®- mixed resin and DAP-mixed resin to cure was 20 seconds, 101 seconds and 160 seconds, with pH of the mixtures were

4, 6 and 9 respectively. As for control samples, the average curing time was 140 seconds and the pH was neutral. The results showed that as the amount of MAP and BP® added into the resin was increased, the curing time was shorter and the pH was lower. Longer curing time and higher pH value were recorded as the amount of DAP added into the resin was increased. This confirmed that the acid based resin, UF cure faster with the decrement of pH value and it shows that fire retardant added into the resin affected the curing time and pH of the resin (refer to table 1).

Table 1 Table shows the curing time and pH of BP®-mixed, MAP-mixed, DAP-mixed and control samples

Samples	pH	Water Absorption (%) ± S.D	Curing Time (s)
MAP 10 %	4	86.29 ± 5.00	20
MAP 8 %	5	162.22 ± 5.00	28
DAP 10 %	9	69.04 ± 6.00	160
DAP 8 %	8	31.86 ± 6.37	150
BP ®10%	6	21.89 ± 4.21	101
BP ®8%	6	21.41 ± 4.00	92
Control	7	202.77 ± 4.00	140

The alkalinity of DAP lengthen the curing time, the acidity of BP® and MAP shorten it. However, it was also expected that the curing time affected by the ammonium chloride added into the resin as a hardener during mixing process. It was well known that the effect of NH_4Cl on UF resin curing is to release H^+ by reacting with free formaldehyde, and then H^+ reacts with OH^- to forms water. The releases of H^+ reduced the pH of the resin; hence make it cure faster (Xing *et al.,* 2006). Therefore, further research can be done to investigate the curing time of DAP and MAP-mixed UF resin without the addition of ammonium chloride as a hardener.

Solubility Test

The average time recorded to dilute 8 % w/w MAP into a beaker of water without heat was 20 seconds. Longer dilution time was recorded as the amount of MAP added increased to 10 % w/w, which was 30 seconds. When heat was applied during the dilution process, the dilution was within 10 seconds for both amounts. For DAP, without application of heat, the chemical was so easily diluted for both amounts within 5 seconds. Different results were obtained for BP®. The chemical started to dilute at the temperature of 70º C and it took 14 seconds to be completely diluted for both amount when the temperature reached 100º C.

Water Absorption Test

The average percentage of water absorbed for the samples that have been mixed with 10% w/w MAP, BP® and DAP is 202.77%, 31.86% and 162.22% respectively. Average water absorption value recorded for resin mixed with 8 % w/w MAP, BP® and DAP is 86.29%, 21.89% and 69.04 %. For control samples lower average value was recorded, that is 21.41% (refer to Table. 1). Between the three fire retardants, DAP was found to be the most active fire retardant in increasing the moisture uptake of the resin, followed with MAP and BP®. Currently, there is still no research on water absorption of fire retardant-mixed UF resin can be found to be used as a comparison for this study.

It has been mentioned by Anthony (1996) that urea formaldehyde is a material which is highly hygroscopic. Therefore, the moisture absorption results summarized in the table above were also affected by the hygroscopic property of the resin. However, based on comparisons made between mixed and control samples; it is clearly shown that MAP, BP and DAP triggered the moisture uptake of the resin. MAP and DAP themselves are naturally chemicals with high hygroscopicity. This was confirmed by Izran *et al.* (2008) and Abdul Rashid *et al.* (1990). They treated particle boards with both phosphorous-based fire retardants (MAP and DAP) and found out that those fire retardants increased the water absorption of the treated particle boards. The water absorption increased when higher amount of MAP, BP® and DAP was used. Bendtsen (1998) treated solid wood with MAP and DAP and it was also found that the fire retardants increased the water absorption of the solid wood.

The results of water absorption recorded for BP® above was contradicted with Izran *et al.* (2008). In his research, he found that BP®-treated particle boards have the highest water absorption value compared to MAP and DAP-treated particle boards. This might be caused the amount of BP® existed in the particle boards is lower than the amount mixed into the resin. Boric acid exists in the formulation of BP® is the key factor in increasing the water absorption ability of the chemical. However, this component cans easily volatize to the surrounding from the sample together with water vapour. It was studied that, different amount of boric acid volatizes at different temperature (Zaidon, 1995). The temperature used for hot pressing BP®-treated particle boards was 180ºC, which is 10ºC higher than the temperature used during the curing time test. This might be the cause that increases the volatilisation of boric from the particle board, compared to the resin, thus created different water absorption rate of both mediums.

All three fire retardant-mixed resin and controls produced odour which minorly irritate the breathing system and cause skin itchiness. This might be caused by the existence of urea in the resin and also in the fire retardants (Anthony, 1996 and Anon,2009a). This is based on the 'pee-like' smell produced during the curing time test.

CONCLUSIONS

MAP, BP® and DAP affected the curing time and water absorption of UF resin. The higher the amount of fire retardant used the higher would be the amount of the water absorbed. However for the curing time test, MAP shortened the curing times but DAP lengthened it. Modification of the resin formulation is crucial to make the resin more stable, even after mixed with MAP, BP® and DAP, because shorter and longer curing time will not only affect the strength of the final products, but it also will affect the production cost and time. Modification can be made on the pH of the resin prior to the addition of fire retardants.

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REARING A SOLITARY GREEN SEA TURTLE (CHELONIA MYDAS) IN CAPTIVITY

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RESEARCH NOTE. Sea turtles are difficult to rear in captivity due to many reasons including fatal bites from other turtles, malnutrition, bacterial diseases, fungal infections and viral diseases (George, 1997). The Borneo Marine Research Institute of Universiti Malaysia Sabah was given a solitary green turtle *(Chelonia mydas)* as part of a rescue and rehabilitation programme on 20 October 2004. The turtle was raised in captivity for a period of approximately 40 months from the age of 20 days in the tanks of the Marine Aquarium and Museum of Universiti Malaysia Sabah (MAMUMS) located in the Borneo Marine Research Institute (Figure 1). The animal thrived in captivity in a 455 L polyurethane tank and its growth was noted to increase immensely once it was moved into its final enclosure, a circular display tank measuring 9 m in diameter with an approximate volume of 90,000 L.

Figure 1. Green turtle *(Chelonia mydas)* reared in captivity at the Aquarium and Museum of Universiti Malaysia Sabah

It was fed daily with fish (*Decapterus* sp.), squid (*Loligo* spp.) and prawns (*Penaeus* spp.). As green turtles are omnivores when young and become herbivores when they reach maturity, plant matter in the form of seaweed (*Sargassum* sp.) and various leafy vegetables ((Choy Sum (*Brassica* chinensis), lettuce (*Lactuca* sativa) and spinach (*Spinacea oleracea*)) were also offered to the turtle, however it showed a preference for canned peas (*Pisum sativum*).

This is the first case study of a sea turtle being raised in captivity as an individual. Most captive-raised turtles are raised in groups in accordance to their clutch size, but stocking density would depend on the size of the enclosure. The emphasis of this paper is the survival of a solitary animal being reared in captivity. Mortality rates of captive-raised turtles can exceed 36% (Palaniappan, 2007).

Captive-raised sea turtles are known to have high growth rates due to their protein-rich diet (Brown *et al.*, 1982; Swingle *et al.*, 1993; Wood and Wood, 1993; Palaniappan, 2007). The protection from predators, controlled water temperatures and lack for space for exercise also contributes to their fast growth. The green turtle raised here was comparable in weight to those being raised in captivity at the Cayman Turtle Farm (Wood and Wood, 1981). Their turtles aged 44 months weighed between 25.1 to 28.7 kg whereas the UMS turtle weighed 28.0 kg at 39 months. This turtle measured 5 cm when it first arrived at the Aquarium and reached 66 cm (curved carapace length) after 39 months of growth.

Captive-reared sea turtles do not have long life spans; therefore this effort of raising a green turtle as a solitary animal is a notable achievement in sea turtle husbandry. This gives hope towards sea turtle conservation as sea turtles from the wild in need of rescue and rehabilitation have hope of surviving in captivity.

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