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Contents

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Hypoglycemic Effect of Extracts of Petai Papan *(Parkia speciosa, Hassk)*

FATHAIYA JAMALUDIN and SUHAILA MOHAMED[1]
Department of Food Science,
Faculty of Food Science and Biotechnology,
Universiti Pertanian Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRAK

Pentadbiran extrak kloroform petai melalui mulut, dapat menurunkan dengan ketara ($p<0.01$) kandongan glukos dalam darah tikus yang di kencing manis oleh alloxan. Tindakan hypoglysaemik ini berkadaran dengan punca kuasa dua dos yang diberi. Tindakan hypoglysaemik adalah mixima selepas 2-5 jam pengambilan ekstrak tersebut melalui mulut dan kekal selama sekurang-kurangnya 24 jam.

ABSTRACT

The oral administration of the chloroform extract of Parkia speciosa *to alloxan-induced diabetic rats produced a significant ($p<0.01$) decrease in blood glucose levels. The hypoglycemic response was approximately proportional to the square root of the dose given. The hypoglycemic activity of the extract reached a maximum 2-5 hours after oral administration of the extract and lasted for at least 24 hours.*

Keywords: *Parkia speciosa*, antidiabetic, hypoglycemic, oral administration, rats, chloroform extract, dose-response

INTRODUCTION

Petai (*Parkia speciosa*) is a Southeast Asian legume of the Mimosae subfamily, whose seeds are consumed as a condiment or vegetable with rice, for its unique Shiitake mushroom-like flavour. When taken in excess it gives a strong onion-like smell, which is excreted by the body in the urine, the sweat and the faeces. Sometimes petai is eaten because it is believed to have anti-diabetic and anti-hypertensive activity.

Petai has been used in traditional medicine for its antibacterial effects on kidney, ureter and urinary bladder. The antibacterial and antifungal compounds were found to be cyclic polysulfides, whose structures were established as 1,2,4-trithiolane, 1,2,4,6-tetrathiepane, 1,2,3,5,6-pentathiepane (lenthionine), 1,2,4,5,7,8-hexathionane and a pentathiocane (Gmelin *et al.* 1981). Dichrostachinic acid, djenkolic acid and thiozolidine-4-carboxylic acid were also identified (Holzman *et al.* 1982). Thiozolidine-4-carboxylic acid has been successfully used experimentally and clinically as an anti-cancer agent (Pandeya 1972). Djenkolic acid has been known to cause blockage of the urinary tubules due to its low solubility, resulting in pain, haematuria and even death. *P. speciosa* seeds also contain significant minerals, vitamins, protein and fat, while having a lower antinutrient content compared to soya bean (Suhaila *et al.* 1987).

This research was undertaken to investigate the hypoglycemic effect of *P. speciosa* on normal and alloxan-induced diabetic rats, because petai is eaten by diabetics for that purpose.

[1]To whom all correspondence should be addressed.

MATERIALS AND METHODS

Preparation of Extracts

Ten kg of fresh petai pods were obtained from the local market. The seeds were separated from the pods. Both portions were air dried, ground to a powder and extracted sequentially and exhaustively with petroleum ether, diethyl ether, chloroform, dichloromethane, ammoniacal chloroform and methanol. The solvents were completely evaporated off with a rotary evaporator to obtain the extracts.

Experimental Procedure

Healthy Sprague Drawley rats of mixed sexes (weighing 200-450 g) were intravenously injected with 60 mg/kg alloxan (2,4,5,6-Tetra oxy pyrimidine) to induce diabetes within 40-48 hours (Lundquist and Rerupa 1967). The dry extracts of petai were orally fed to 24-hr-fasted normal and alloxan-induced diabetic rats at a dose level in the range of 25-500 mg extract/kg BW (body weight), together with 1 g glucose/kg BW of rat. Coadministration of glucose with the extract was done to cause hyperglycemia. Both diabetic and normal rats treated orally with 5 ml saline and 1 g glucose/kg BW were observed for comparison. Blood samples were taken hourly for the first 11 hours and again 24 hours after the administration of the extracts. Blood was obtained from the tail vein by using heparinised microhematocrit capillary tubes (Riley 1960).

Analysis of Blood Glucose

The plasma glucose level was determined by glucose oxidase method (Roche Glucose test kit No 07 1011 3) where D-glucose is specifically oxidised to gluconic acid and hydrogen peroxide by glucose oxidase. The generated hydrogen peroxide converts O-dianisidine, by the catalytic action of peroxidase to the red-brown semiquinone. The colour intensity is directly proportional to the glucose concentration and is measured spectrophotometrically. 0.02 ml of serum was used for glucose assay and compared with 0.02 ml standard D glucose solution.

Statistical Analysis

The data were statistically analysed using analysis of variance (ANOVA), Duncan's multiple range test (DMRT) and regression analysis on MSTAT computer program.

RESULTS AND DISCUSSION

Results showed that only the chloroform extracts (1 g/kg body weight) from both the empty pods and seeds of petai had a strong hypoglycemic activity on diabetic rats *(Fig. 1)*. Blood glucose level at time zero is the blood glucose level just after the oral administration of extracts/saline and glucose. ANOVA analysis showed significant differences between chloroform extracts of both the seeds and pods ($p<0.01$), and extracts from other solvents

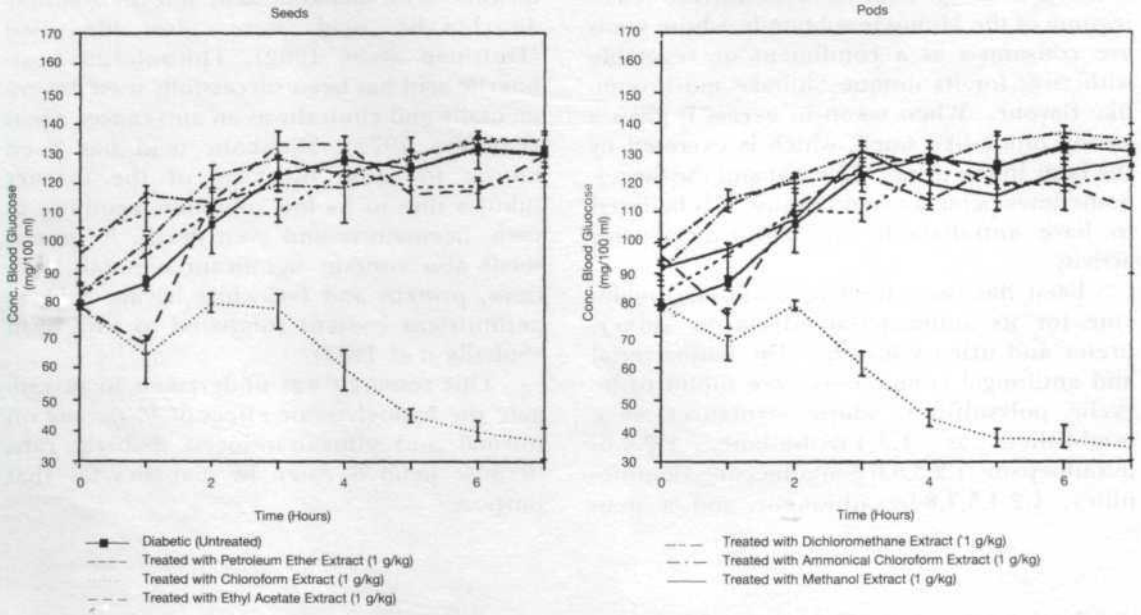

Fig. 1. Effect of different chemical solvents extracts of P. speciosa *on blood glucose levels in alloxan-diabetic rats. Data are means ± SE (n = 4)*

(1 g/kg body weight) or the control (treatment with saline). Further work therefore concentrated only on the chloroform fraction.

Fig. 2 shows that there was insignificant increase in the blood glucose levels of normal rats fed with 0.4 g ground seeds or pods together with 1 g glucose/kg body weight. The normal rats had an average blood glucose content of 124 mg/100 ml, while the alloxan diabetic rats had an average blood glucose level of 379 mg/100 ml after ingesting 1 g glucose/kg body weight.

The blood glucose level of alloxan diabetic rats was reduced by 36±6 % to 288 mg/100 ml with the oral treatment of 0.4 g/kg BW pericarp (pod), and by 57±6 % to 236 mg/100 ml after the oral treatment with 0.4 g/kg BW petai seed. The treatment could be seen to take effect within less than an hour and lasted for at least 24 hours. The maximum fall was observed 2 hours after oral administration. However, there was an initial rise in blood glucose level between 0-3 hours, showing that the glucose was rapidly absorbed from the alimentary canal and that the extract of petai took effect only two hours after ingestion. The blood glucose level of healthy and diabetic rats fed with saline plus 1 g/kg BW glucose is shown for comparison.

The seed had a higher activity than the pericarp. *Fig. 3* shows the dose-response relationship of petai seed on blood glucose level in diabetic rats. A dose of 25 mg/kg BW decreased the blood glucose by 24±4 %, yet a 4-fold increase in dosage (100 mg/kg BW) only decreased the blood glucose by 43±5 %. Increasing the dosage 20-fold (500 mg/kg BW) decreased the blood glucose by 77±12 %. Further increasing the dosage (3 g seed/kg BW) decreased the blood glucose level by 116±12 % i.e. bringing the glucose level below that of a normal healthy rat.

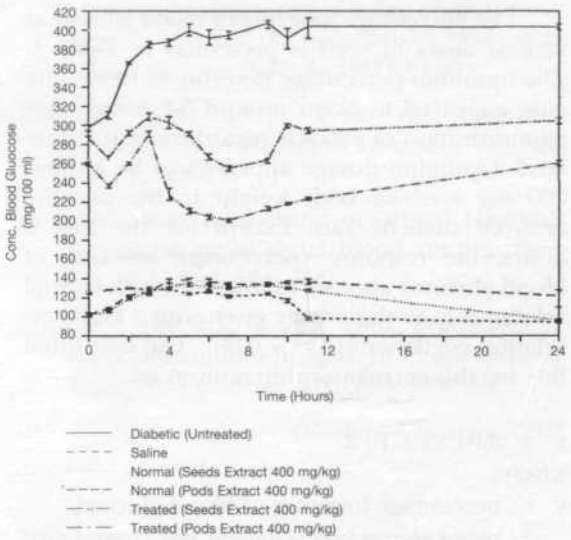

Fig. 2. Effect of chloroform extracts of P. speciosa *on normal and diabetic rats. Data are means ± SE (n = 4)*

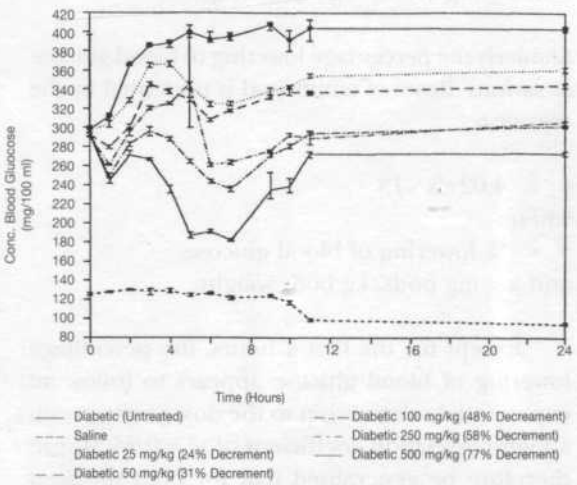

Fig. 3. Dose-response relationship of fresh and ground seeds on blood glucose levels of alloxan-diabetic rats. Data are means ± SE (n = 4)

TABLE 1
Reduction in blood glucose (%) of
diabetic rats following the administration of chloroform extract of petai seeds

dose hr (mg/kg body wt)	50	100	150	200	250	300	350	400	450	500
1	25.41	51.76	53.17	55.52	56.47	57.41	58.35	59.29	58.35	57.41
3	20.70	30.11	32.94	36.70	37.64	37.64	38.58	39.05	43.76	48.94
5	25.4	34.82	43.29	50.82	55.52	59.29	64	67.76	72	75.76
8	25.41	46.11	53.17	60.23	64	73.88	66.35	68.70	69.17	70.11
11	13.17	30.11	36.70	44.23	47.05	49.41	51.76	53.64	44.70	36.70
24	8.941	21.64	27.29	33.88	34.35	34.82	35.76	36.70	34.82	33.88

The percentage lowering of blood glucose at various doses of seed is presented in Table 1. The optimum percentage lowering of blood glucose appeared to occur around 5-8 hours after administration of extracts regardless of the dose level. Optimum dosage appeared to be around 200 mg seed/kg body weight in the alloxan-induced diabetic rats. Except for the first 2 hours, the response (percentage lowering of blood glucose) appears to follow an exponential relationship to the dosage given with a high correlation coefficient of $r^2 = 0.99$. The best fitted line for this correlationship is given as:

$$y = 3.01 \sqrt{x} + 10.2$$

where:

y = percentage lowering of blood glucose,

$$= \frac{(\textit{blood glucose level of diabetic rats - treated rats}) \times 100}{(\textit{blood glucose level of diabetic rats - healthy rats})}$$

and x = mg seeds/ kg body weight.

Similarly the percentage lowering of blood glucose at various doses of empty pod is presented by the equation

$$y = 4.02 \sqrt{x} - 13$$

where:

y = % lowering of blood glucose,

and x = mg pods/kg body weight.

Except for the first 4 hours, the percentage lowering of blood glucose appears to follow an exponential relationship to the dosage given with a high correlation coefficient of $r^2 = 0.94$. It can therefore be generalised that for both the seed and pericarp, the response is approximately proportional to the square root of the dose given. The time taken for the pericarp to take effect and the duration of the hypoglycemic activity are shown in *Fig. 4*. The pericarp had a lower activity than the seed. At 25 mg/kg BW there was no significant activity. At 50 mg/kg BW the lowering of blood glucose was 18±4 % and the activity at 100 mg/kg and 250 mg/kg was 31±5 % and 47±5 % respectively. This is about half the activity of the seed.

The fact that the blood glucose response to petai seeds and pods is square root to the dose may indicate that the mechanism of action of the

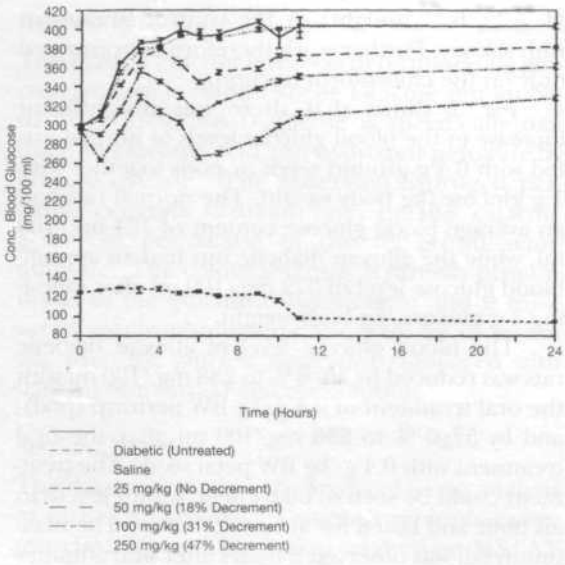

Fig. 4. Dose-response relationship of fresh and ground empty pods on blood glucose levels of alloxan-diabetic rats. Data are means ± SE (n = 4)

active compounds in petai is peripheral. This is based on comparison of dose response curves of peripherally-acting compounds to centrally-acting ones (those causing the pancreas to increase insulin production and release). Peripheral-acting compounds act directly on all the cells in general, enabling more glucose to enter the cells. Chemical studies on the active compounds of petai showed them to be sterols (results to be published) which can readily affect the lipoprotein part of cell membranes. Further work is being carried out to determine the mechanism of action.

Even though the activity of the pericarp (empty pod) and mesocarp (testa) are half of that from the seed, extraction of compounds from the empty pod is viable because it constitutes 57 % of the whole pod and only the seeds are normally eaten while the outer skin is less palatable although edible. In Malaysia, canned petai seeds with anchovies and chilli sauce are available in the market. The empty pods are therefore a waste product which can be used as a raw material for the extraction of hypoglycemic material.

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Growth Performance and Gonad Development in Diploid and Triploid *Clarias batrachus* (Linnaeus)

S.S. SIRAJ, S. K. DAUD and K. J. ANG
Department of Fish Biology and Aquaculture
Faculty of Fisheries and Marine Science
Universiti Pertanian Malaysia
43400 UPM, Serdang, Selangor, Malaysia.

ABSTRAK

Sibling penuh ikan keli kayu, Clarias batrachus, *diploid dan triploid dipelihara dalam tangki gentian kaca segi empat mulai umur tiga minggu. Prestasi pertumbuhan ikan diploid dan triploid dibandingkan dengan diberi makanan yang mengandungi 30% protein. Pada akhir tempoh ujikaji ini kadar pertumbuhan di antara diploid dan triploid didapati tiada perbezaan bererti (P>0.05). Walau bagaimanapun gonad ikan triploid tidak begitu berkembang. Ovari ikan triploid mengandungi bilangan oosit yang kurang dan beberapa oosit matang yang abnormal. Testis ikan triploid mengandungi bilangan tubul seminiferus yang kurang dan jumlah tisu perantara yang bertambah.*

ABSTRACT

Full siblings of diploid and triploid walking catfish, Clarias batrachus, *were reared in rectangular fibreglass tanks starting at the age of three weeks. Growth performances of diploids and triploids were compared by feeding with a 30% protein diet. At the end of the study period growth rate was found to be insignificantly different (P>0.05) between the diploid and the triploid. However, triploid fish had poorly developed gonads. Triploid ovaries contained fewer primary oocytes with some abnormal maturing oocytes. Triploid testes contained fewer seminiferous tubules and a larger amount of connective tissue.*

Keywords: diploid, triploid, growth, gonad development

INTRODUCTION

The catfish, *Clarias batrachus* and *C. macrocephalus*, are very popular and constitute some of the most important freshwater fish in Malaysia. They are utilised not only for fish farming but also for inland fisheries. They attain a size of above 200 g after four to six months of culture in earthen ponds. In Malaysia spawning induction in walking catfish has been described by Thalathiah (1986) and Cheah *et al.* (1990) but there is no information on chromosome manipulation work on this fish. Triploidy has been successfully achieved in *C. macrocephalus* and *C. gariepinus* (Richter *et al.* 1987; Vejaratpimol and Pewnin 1990). Recently, triploid *C. batrachus* were produced by cold-shocked treatments of the inseminated eggs (Manickam 1991; Siraj *et al.* 1992). The interest is especially focused on triploidy, as triploid fish have been assumed to be sterile and potentially can avoid the growth depression, poorer feed conversion and survival, losses which are associated with sexual maturation in normal fish (Purdom 1976; Thorgaard and Gall 1979; Gervai *et al.* 1980).

Gonad development and fertility in induced triploidy and higher degrees of ploidy are known for a number of amphibian species (Frankhauser and Humphrey 1950; Bungenberg De Jong 1957), but comparatively few studies have been made on fish, probably because of the difficulties of inducing and rearing polyploid fry. Experimentally-induced triploids have been reared in a variety of fish (Swarup 1959a,b; Purdom 1972; Valenti 1975; Wolters *et al.* 1982; Chourrout *et al.* 1986). In some of these species polyploidy appeared to be associated with a marked disruption of gonad development; either lacking or undeveloped, although some fish within the normal diploid range for nuclear cell were also sterile. Thus, triploids might be more valuable and profitable to raise than diploids.

This study was conducted to examine the effects of triploidy on the growth performance and gonad development of *C. batrachus*.

MATERIALS AND METHODS

Three-week-old full siblings of triploids (obtained from cold shock treatment inseminated eggs immersed in water at 5°C and 10°C, for a duration of 3, 5 and 10 min and assessed by the size of erythrocytes major axis (Siraj *et al.* 1992)) and normal diploids were reared in 500 l rectangular fibreglass tanks containing 200 l of water, equipped with biological filters and aeration. Fifty fish were stocked in each tank with three replicates per treatment (both diploid and triploid). Fish were fed with a 30% protein diet two times a day and uneaten feed was siphoned out. Water quality parameters, such as temperature, pH, dissolved oxygen, carbon dioxide and nitrite were monitored during the experimental period. The rearing experiment was terminated at the end of five months since more than 50% of the fish died. All fish were weighed to the nearest 0.1 g. Feed conversion was calculated from the weight of feed consumed divided by the weight of fish produced (wet weight). Growth performance and feed conversion rate were analysed using statgraphic statistical programme.

At the end of ten months ovaries and testes from five females and five males from each group were removed and immediately fixed overnight in Bouin's solution. The gonads were dehydrated in a series of alcohol and mounted in paraffin wax and stained following Drury and Wallington (1980). Transverse sections from both ends of each gonad were cut at 5-6 µm in thickness and stained with hematoxylin and counterstained with eosin. Slides of both ovary and testes from diploid and triploid *C. batrachus* were examined for their cell structure and stages, and photographed.

RESULTS

Table 1 gives mean total length and weight, and feed efficiency of diploid and triploid *C. batrachus* from three weeks to five months of age. At 5 months old the values were 148.09±2.05 mm, 26.40±1.24 g and 1.47 for diploid fish and 147.60±2.25 mm, 25.91±1.25 g and 1.44 for triploid fish, respectively. There was no significant difference ($P>0.05$) between diploid and triploid *C. batrachus* in weight, total length and feed conversion rate up to five months of growth. Water quality parameters such as temperature, pH, dissolved oxygen, carbon dioxide and nitrite were within the physiological conditions.

The bilobed testes of diploid walking catfish at an age of ten months were elongated and the posterior end of each lobe was fused to form a single *ductus efferen*. The anterior region of the testes contained a series of seminiferous tubules where spermatogenesis takes place. Reduced size of the posterior end of the testes suggests that fewer seminiferous tubules develop in this region. General structure of the triploid walking catfish testes *(Plate 1B)* was almost similar to that of diploid *(Plate 1A)* except that the size was smaller, and coiled seminiferous tubules at the anterior region were fewer and irregular in shape.

TABLE 1.
Mean (± SD) weight, total length and feed conversion rate for diploid and triploid walking catfish *Clarias batrachus* at 5 months.

Variable	Diploid		Triploid	
	Mean weight (g)±SD	Mean total length (mm)±SD	Mean weight (g)±SD	Mean total length (mm)±SD
3 weeks	2.10±0.90	60.98±1.01	1.65±0.06	57.07±0.71
5 months	26.40±1.24	148.09±2.05	25.91±1.25	147.60±2.25
Feed conversion ratio (at 5 months)	1.47		1.44	

A histological section of testes from diploid catfish revealed the seminiferous tubules were encircled by spermatogenesis epithelium of the same thickness. The lumen of tubules were filled with many spermatozoa *(Plate 2A)*. In the triploid testes there was irregular thickness of the spermatogonia epithelium, and an increased amount of connective tissue *(Plate 2B)*. This suggests that a great proportion of the spermatocytes and spermatids degenerate. Similar observations were reported by Lincoln (1981) and Wolters *et al.* (1982) in plaice and channel catfish respectively.

The size of triploid ovaries was about one-fourth of the diploid ovaries *(Plate 3)*. Various stages of developing oocytes were observed in the histological section of the diploid ovaries *(Plate 4A)*. Strong basophilic cytoplasm and light stained nuclei were found in immature and

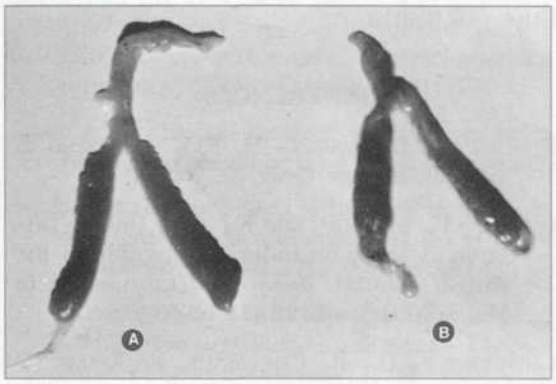

Plate 1 Diploid (A) and triploid (B) testes of walking catfish. Clarias batrachus *(2x) at 10 months.*

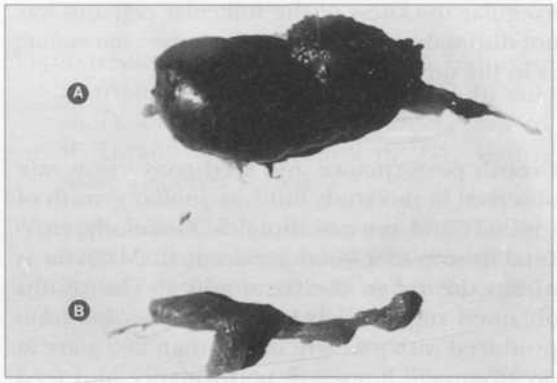

Plate 3 Ovaries of diploid (A) and triploid (B) of walking catfish Claris batrachus *(2x) at 10 months.*

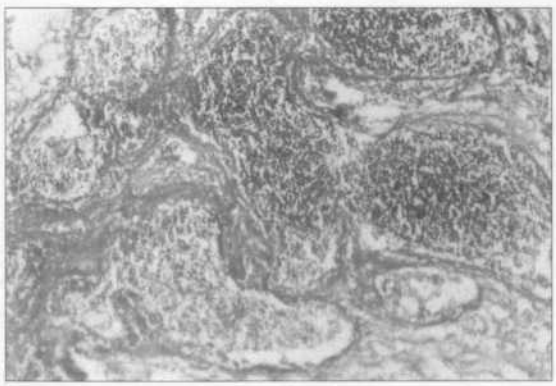

Plate 2A Histological section of testes of diploid walking catfish Clarias batrachus *(200x)*

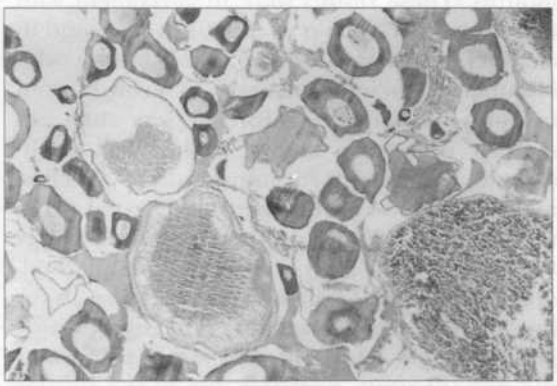

Plate 4A Histological section of ovary of walking catfish Clarias batrachus *(400x)*

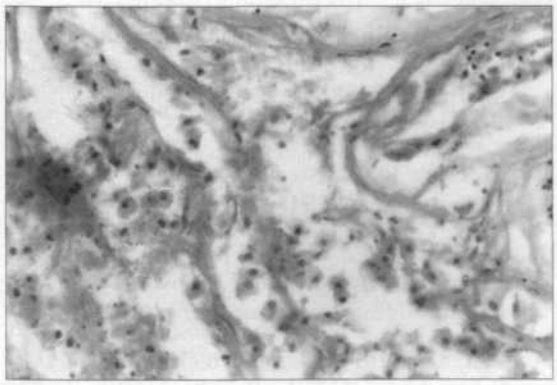

Plate 2B Histological section of testes of diploid walking catfish Clarias batrachus *(400x)*

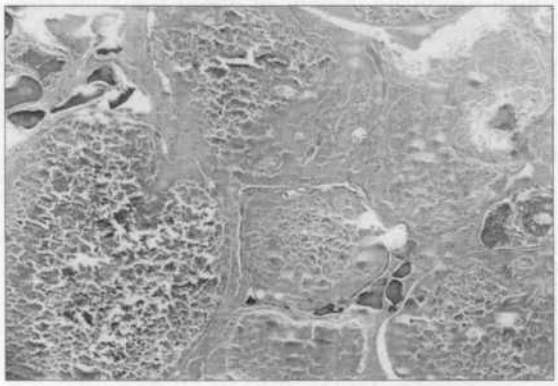

Plate 4B Histological section of ovary of triploid walking catfish Clarias batrachus *(400x)*

maturing oocytes of the diploid catfish. In contrast the triploid ovaries had fewer oocytes in different developmental stages. Many of the maturing oocytes had poorly defined nuclear membranes and highly granular cytoplasm which was different from the normal stage VI oocyte in the diploid ovaries. Few primary oocytes were also present in the ovary of the triploid catfish *(Plate 4B)*. The triploid oocytes showed an irregular thickness of the follicular cell and was not distinctly differentiated from the *zona radiata* as in the diploid catfish.

DISCUSSION

Growth performance and feed conversion rate observed in this study indicate similar growth of triploids and normal diploids. Generally, marketable size of *Clarias batrachus* in Malaysia is within the range of 200 to 250 g. The results obtained suggest that triploid *Clarias batrachus* produced with a weight of less than 200 g are at no advantage in growth performance and feed conversion rate. The advantage could be felt if the marketable weight is more than 500 g at ten months. These results were in agreement with work done by Gervai *et al.* (1980) in carp, and in channel catfish by Wolters *et al.* (1982) who found no significant increase in growth from triploidy in immature fish of less than six months' growth. In theory, triploid fish would expend less energy for reproductive development during the normal period of sexual maturation than diploids and therefore show better feed conversion. Thus, the lack of gonad development in triploids provides an explanation for the better feed conversion and significantly greater weight. However, this was not shown in the present study, probably due to the shorter period of rearing and the marketable size of the fish which is less than 200 g. The triploid males which produced gonads of about the same size as in diploids give no advantage in term of growth performance and feed conversion rate. Further repeated experiments are required to verify the theory as proved by studies on plaice-flounder hybrids, *Pleuronectes platessa X Platichthys flesus*, in rainbow trout, *Salmo gairdneri*, and in channel catfish, *Ictalurus punctatus* (Purdom 1976; Thorgaard and Gall 1979; Wolters *et al.* 1982). Further research is also needed to look into the economics of producing triploid and other chromosomal manipulated walking catfish which could be of greater value to the fast-developing fisheries industry in the country.

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The Life-cycle of *Biosteres persulcatus* with Reference to Adults' Reproductive Capacity on Eggs of Carambola Fruit-fly

A.G. IBRAHIM, I.P. PALACIO and ROHANI IBRAHIM
Department of Plant Protection,
Faculty of Agriculture,
Universiti Pertanian Malaysia,
43400 UPM, Serdang, Selangor Darul Ehsan, Malaysia.

ABSTRAK

Kajian telah dijalan di makmal (26.5°C ± 1.5°C) bagi mengkaji edaran hidup, Biosteres persulcatus *Silvestri, parasitoid pada larva lalat buah* (Bactrocera (B) *sp. near* Bactrocera dorsalis *A). Terdapat 4 peringkat larva berasaskan kepada saiz peralatan mulut. Penjelmaan larva yang pertama berlaku dalam kepompong perumah yang baru dibentuk. Jumlah masa perkembangan kedewasaan jantan dan betina ialah 16.3 ± 0.80 hari dan 17.1 ± 0.80 hari. Purata keupayaan pembiakan semasa hidup ialah 67 ± 3.5 biji telur.*

ABSTRACT

A study of the life-cycle of Biosteres persulcatus *Silvestri, a larval parasitoid of (*Bactrocera (B) *sp. near* Bactrocera dorsalis *A), was conducted in the laboratory (26.5°C ± 1.5°C). There are 4 larval stages as indicated by the sizes of the mouthhooks. The first larval moult occurred in the newly-formed puparium of the host. The entire developmental period from egg to adult emergence for male and female was 16.3 ± 0.80 days and 17.1 ± 0.80 days respectively. The average reproductive capacity during the life span was 67 ± 3.5 eggs.*

Keywords: *Biosteres persulcatus* Silvestri, larval development, reproductive capacity, carambola fruit-fly

INTRODUCTION

The carambola fruit-fly *Bactrocera* (*Bactrocera*) sp. near *Bactrocera dorsalis* A (Diptera : Tephritidae) (White and Elson-Harris 1992) is of economic importance because of its climatic tolerance, geographical distribution and diversity of hosts. The *dorsalis* complex comprises many species (Drew 1989).

Bactrocera dorsalis complex has several natural enemies (Clausen *et al.* 1965, Bateman 1972). In Hawaii van den Bosch and Haramoto (1953) attributed the success of *Biosteres persulcatus* over other opiine parasitoids to its ability to inhibit physiologically the development of other parasitoids in their hosts. This parasitoid, *Biosteres persulcatus* which was originally from South Asia was introduced into Hawaii during the 1935-1936 project on biological control (Wharton 1989). Recently, effective trapping methods of *Biosteres persulcatus* in the field have been developed in Hawaii (Vargas *et al.* 1991; Messing and Wong 1992).

In Malaysia, seven species of opiine parasitoids were recorded from *Bactrocera dorsalis* complex (Rohani 1986). The effective parasitisation of *Bactrocera dorsalis* complex depends on the geographical location of the orchards (Ooi 1984; Vijaysegaran 1984, 1991; Palacio 1991). Therefore in an effort to evaluate the potency of *Biosteres persulcatus* in regulating the populations of *Bactrocera* (B) sp. near *Bactrocera dorsalis* in tropical fruit orchards, knowledge of the biology of the parasitoid is important. This work investigates the life-history of *Biosteres persulcatus* and the reproductive capacity of the adults.

MATERIALS AND METHODS

The biological studies were conducted under laboratory conditions of 26.5°C±1.5 °C and 72.5 ±7.5% RH at the Department of Plant Protection, Universiti Pertanian Malaysia. The field trial was conducted at the university farm, Puchong, which has an orchard for production of carambola fruits.

For life-cycle study, slices of ripe guava var. Kampuchean, each measuring 4 x 5 x 1 cm were placed in a shallow pan (5 cm diam.) exposed to approximately 2000 females of *Bactrocera* (B) sp. near *Bactrocera dorsalis* A for an hour of oviposition. The first-instar larvae of the fruit-flies were then exposed to 100 females of *Biosteres persulcatus* in a cage measuring 20 x 20 x 20 cm for 3 hours. To determine the incubation period of

parasitoid eggs, 100 parasitised *Bactrocera* larvae were dissected under a stereomicroscope commencing 22 hours after exposure to the parasitoids. This was done at hourly intervals until all the parasitoid eggs had completed the incubation period. After hatching, another 100 parasitised hosts were dissected daily until all the parasitised larvae had pupated. The parasitised pupae were individually weighed and recorded for adult emergence.

To determine reproduction of *Biosteres persulcatus*, pairs of newly-emerged male and female parasitoid adults were confined separately in plastic cages measuring 4 cm tall and 4 cm diam. Each pair of *Biosteres persulcatus* was offered daily a slice of guava fruit (2 x 2 x 1 cm) containing at least 50 first-instar larvae of carambola fruit-fly. An undiluted commercial honey was regularly streaked on the inner wall of the cage to serve as food for adult parasitoids. Hosts offered to 20 pairs of adult parasitoids were dissected daily to determine the fecundity of the parasitoids. The hosts offered to another batch of 20 parasitoids were reared on an artificial diet until the emergence of the parasitoids. Ten female parasitoids of known age were dissected daily to determine the number of mature eggs in the ovaries.

Longevities of adult parasitoids when kept with and without hosts were measured. Twenty pairs of parasitoids were kept with fruit slices containing first-instar larvae of fruit-fly and another 20 pairs were only fed with diluted honey (10%). Their survival rate was recorded.

RESULTS AND DISCUSSION

Larval Development

Table 1 shows the entire developmental period of the parasitoids. The egg of *B. persulcatus* is elongated with rounded ends measuring 0.68 ± 0.005 mm long and 0.09 ± 0.002 mm wide when newly laid. A fully-incubated egg measured 0.76 ± 0.007 mm long and 0.17 ± 0.002 mm wide. The mean incubation period of eggs was 27.0 h with 95% hatchability.

TABLE 1

Developmental parameters of *Biosteres persulcatus* Silvestri at 26.0 ± 1.5°C and 72.5 ± 7.5% RH

Stage[a]	Duration (Days)		Survival (%)	
	Range	Mean	Range	Mean
A. Egg:	0.96 – 1.46 (23 – 35 hr)	1.12 (27.01 hr)	93.00 – 98.00	95.46
B. Larva:	9.00 – 13.00	10.96	88.00 – 96.00	91.00
I:	4.00 – 7.00	5.61	after hatching	
II:	6.00 – 9.00	7.89	after oviposition	
III:	7.00 – 10.00	8.46	after oviposition	
IV:	8.00 – 13.00	10.10	after oviposition	
C. Pupa:				
Female:	5.00 – 6.00	5.60	82.00 – 93.00	88.00
Male:	5.00 – 6.00	5.40	82.00 – 92.00	87.80
D. Entire Development:				
Female:	16.00 – 20.00	17.14	67.11 – 87.49	76.44
Male:	15.00 – 19.00	16.26	167.11 – 86.55	76.27
E. Sex Ratio:	1.10 female : 1 male			

[a]Determined from hourly dissection of 100 samples of parasitised hosts starting 22 h after oviposition for egg and daily for the succeeding immature stages.

The first instar is hymenopteriform with heavily sclerotised mandibles measuring 0.05 ± 0.002 mm long and 0.03 ± 0.001 mm wide. *(Fig. 1)*. The newly-hatched larva measured 0.76 ± 0.007 mm at the early stage increasing to 2.00 ± 0.05 mm at its later stage. The average period for first instar is 5.6 days.

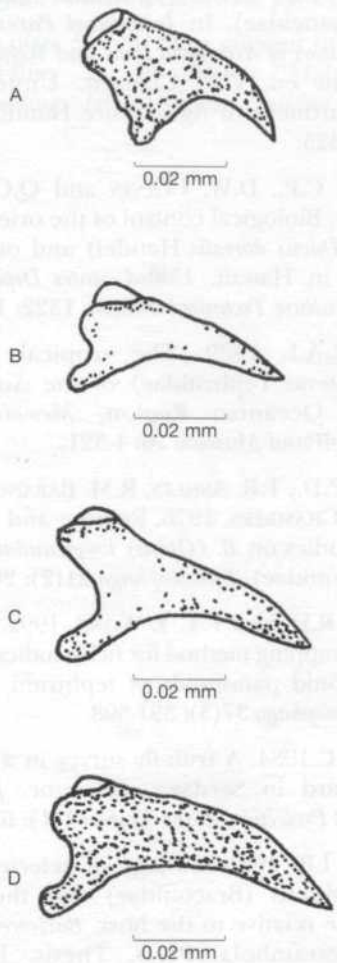

Fig. 1. Mandibles of first through larval instars of Biosteres persulcatus *Silvestri (A – D respectively)*

The second-instar larva is grub-like and the mandibles are unsclerotised. The larva measured 3.12 ± 0.005 mm long and 0.91 ± 0.003 mm wide and lasted one day at most in the newly-formed puparium of the fruit-fly. The third instar is similar to the second instar except that it had increased in size to 4.3 ± 0.004 mm long and 1.4 ± 0.002 mm wide. It lasted for at most one day only and at this stage was yellowish white.

The fourth instar is of similar colour to the third instar but it has numerous spines and 9 pairs of spiracles. The mandibles are heavily sclerotised measuring 0.08±0.002 mm long and 0.3±0.002 mm wide, with incisors curved and acute. The larva measured 5.62±0.07mm long and 2.07±0.05 mm wide and lasted 4 days. The prepupa is dirty white. Inside the host puparium, the pupa is enveloped by a paper-like cocoon. The exuviae of the fourth instar is attached along the apical part of the antennae of the male or ovipositor of the female.

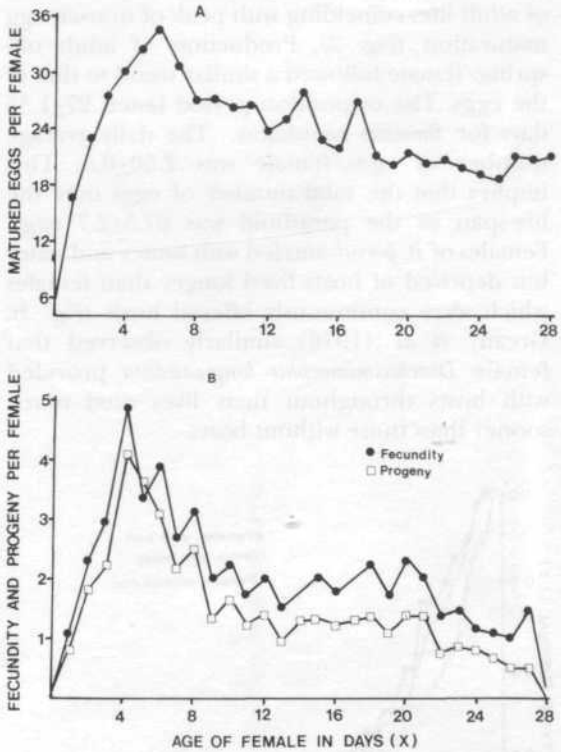

Fig. 2. Daily mean of mature eggs (A); and fecundity and progency production (B) of Biosteres persulcatus *Silvestri (Based on 10 females for A, and 20 pairs for B)*

The pupa is yellowish brown to piceous depending on the age. The female pupa measured 4.8±0.05 mm long and the male 4.7±0.05 mm. The average developmental period for male and female pupae was 5.6±0.6 days and 5.4±0.05 days respectively. The entire developmental period from egg to adult emergence averaged 17.1± 1.5 days for female and 16.2±1.5 days for male. The overall survival was comparable for both sexes, 76.4%±0.75 for females and 76.2%±0.75 for males with a sex-ratio of 1 : 1.

The newly-emerged adult has a reddish brown appearance. The ovipositor shaft is brown with swollen and trisinuate apex. Female adults measure 5.13±0.07 mm long from head to tip of abdomen and 1.22±0.02 mm wide. The male external genitalia is brownish and the aedegus dorsolventrally flat (Palacio *et al.* 1992).

Reproduction and Longevity

Pairs of male and female *Biosteres persulcatus* commenced mating and oviposition on the same day they emerged from pupae. The highest daily mean fecundity/female occurred on the 4th day of adult life, coinciding with peak of ovarian egg maturation *(Fig. 2)*. Production of adult offspring/female followed a similar trend to that of the eggs. The oviposition period lasted 27±1.35 days for *Biosteres persulcatus*. The daily average number of eggs/female was 2.50±0.5. This implies that the total number of eggs over the life-span of the parasitoid was 67.5±2.7 eggs. Females of *B. persulcatus* fed with honey and water but deprived of hosts lived longer than females which were continuously offered hosts *(Fig. 3)*. Greany *et al* .(1976) similarly observed that female *Diachasmimorpha longicaudata* provided with hosts throughout their lives died much sooner than those without hosts.

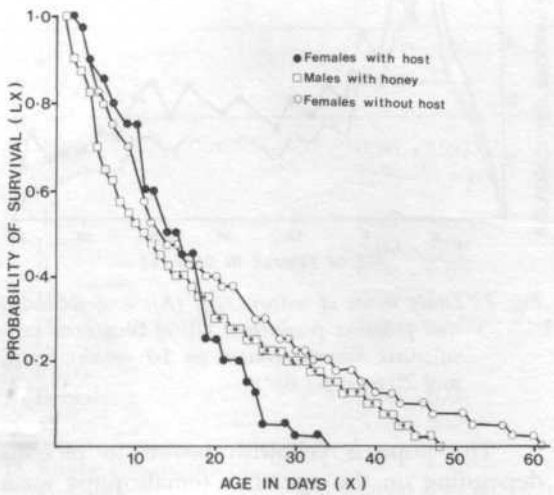

Fig. 3. Adult survival of Biosteres persulcatus *Silvestri in relation to availability of the host (Based on 40 adults each)*

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Water Relations, Stomatal Responses and Physiological Changes of *Lansium domesticum*

MOHD RAZI ISMAIL, MOHD IDRIS Z.A and RUZIAH SALLEH
Department of Agronomy and Horticulture
Faculty of Agriculture
Universiti Pertanian Malaysia
43400 UPM, Serdang, Selangor, Malaysia

ABSTRAK

Pengaruh tegasan air terhadap pertumbuhan, kaitan air dan perubahan fisiologi pokok Lansium domesticum *muda telah dikaji. Terdapat pengurangan yang signifikan terhadap jisim kering daun, batang dan akar apabila berlaku tegasan air. Kandungan air relatif telah didapati menurun tetapi densiti stomata dan kandungan klorofil meningkat dengan tegasan air. Peningkatan densiti stomata walaubagaimanapun tidak memperbaiki respons stomata oleh kerana rintangan stomata didapati meningkat apabila berlaku tegasan air. Peningkatan rintangan stomata menyebabkan pengurangan kadar fotosintesis. Rintangan stomata bagi pokok yang diberi tegasan yang ketara tidak pulih ke nilai yang sama dengan pokok yang diberi pengairan berterusan selepas air dibekalkan semula. Rintangan stomata juga didapati rendah pada jenis langsat dibandingkan dengan dokong menunjukkan langsat mempunyai mekanisma kawalan stomata yang baik sewaktu tegasan air.*

ABSTRACT

The effects of water stress on growth, water relations and physiological changes of young Lansium domesticum *plants were investigated. There was a significant reduction in leaf, stem and root dry weight with increasing water stress. Relative water content was reduced but stomatal density and chlorophyll content were increased with water stress. An increase in stomatal density of plants subjected to water stress did not improve stomatal functioning as stomatal resistance was greater in these plants. This contributed to the reduction in leaf photosynthesis rate. Stomatal resistance of severely stressed plants did not reach a similar level as plants watered continuously after rewatering. Stomatal resistance of langsat was lower than dokong plants indicating that langsat exhibited a better stomatal control than dokong plants.*

Keywords: *Lansium domesticum*, growth, relative water content, stomatal resistance, stomatal density, photosynthesis rate

INTRODUCTION

Lansium domesticum Jack is native to the Malay Peninsula, the Philippines and Java where it is widely distributed and grown. The plant is classified under the order Meliales in the Family Meliaceae. The tree has been well described (Ochse *et al.* 1961; Bamroongrungsa and Yaacob 1990). There are three different types of *Lansium* which are commonly grown in Malaysia, namely langsat, duku and dokong (subtype of duku-langsat). In Malaysia, there is an increasing interest among government agencies and the private sector in large-scale production of duku and dokong especially. Although *Lansium* only contributes 0.1% of export value for tropical fruits in Malaysia, there is a tendency for production to rise in the future due to increasing demand from local and international markets. (Malaysian Fruit Industry Directory 1989/1990). Data on the cultivated area of dokong in Peninsula Malaysia (Table 1) show that the crop has yet to be exploited. Further information on agroclimatic requirements needs to be gathered.

Loam or sandy soil with sufficient organic matter content, good but moist drainage, is suitable for growing the crop. It has been estimated that 150-200 days of rain per annum are required for the crop (Bamroongrungsa and Yaacob 1990). There is no physiological basis for this recommendation. In most crops, favourable microclimatic conditions from the nursery phase to establishment in the field are vital for growth and plant development. It is generally acknowledged that water is of utmost importance to sustain a high percentage of survival at transplanting. It is a well-known fact that water deficiency will affect plant growth and development. There is a lack of information on the impact of water avail-

ability on growth and other plant processes in *Lansium*. These studies have been conducted to understand the physiological processes of *Lansium* plants in response to water stress. A comparative study on the stomatal resistance of dokong and langsat was also conducted.

TABLE 1
Cultivated area of *Lansium domesticum*

Lansium type	Area (ha)
Dokong	186
Langsat	2803
Duku	68

Source: Ministry of Agriculture Malaysia (1988)

MATERIALS AND METHODS

The experiments were conducted in the Greenhouse Unit, Faculty of Agriculture, Universiti Pertanian Malaysia, Serdang, Selangor. The mean daily air temperature ranged between 24.6°C and 33.6°C. The relative humidity ranged between 42% to 78% RH. The plants were grown in pots containing 17 kg mixture of top soil: organic matter: sand in the ratio 3:2:1.

A cyclical water stress was imposed on five-month-old dokong plants for 3, 6, 12 and 24 days. After each drying cycle was completed, plants were watered to field capacity. Soil was maintained at the field capacity for the control plants (by gravimetric method). The experiment was conducted in a completely randomized design with 4 replicates, 2 plants assigned for each replicate.

In another experiment, the stomatal response during recovery phase was determined. Plants were stressed by withholding water for 6 and 24 days and then rewatered. Five plants were used for each of the treatments in this study. Variations in air temperature, vapour pressure deficit (VPD) and intercepted radiation were monitored when the measurements of stomatal resistance were made. Another set of dokong and langsat plants consisting of 10 plants each were planted in pots containing the same soil mixture of 3:2:1, as mentioned above. These plants were used to compare the stomatal response when exposed to water stress. Five plants from each group were stressed for 6 and 24 days, and another five plants were watered to field capacity.

For vegetative growth measurements, stem diameter (7 cm from the base) was recorded using a Vernier caliper. Plant height was recorded fortnightly and the data presented as the cumulative increment of height. Leaf area index (LAI) was recorded using a plant canopy analyser (Model LAI-2000 LiCor, Nebraska, USA) at intervals of two weeks. Leaf area was determined using a leaf area meter (Delta-T Cambridge, UK) at final harvest. Root volume was recorded by the displacement method. Dry weight of leaf, stem and root was determined at final harvest, i.e. 68 days after commencement of the treatments. Plant tissues were oven-dried at 105°C for 16 hours.

Relative water content was determined according to the method by Weatherley (1950). Ten leaf discs of 10 cm^2 from each replicate were weighed for fresh weight and floated on distilled water for 14 hours to determine their turgid weight. Leaf discs were oven-dried for 6 hours at 85°C for dry weight determination. Relative water content was calculated from the following equation:

$$\text{Relative water content (RWC)} = \frac{\text{Fresh wt - Dry wt}}{\text{Turgid wt - Dry wt}} \times 100$$

Ten leaf discs were also sampled for chlorophyll determination. Chlorophyll was extracted using 95% ethanol, and the determination was done according to the procedure by Nose (1987).

Stomatal density was recorded using the methods described by Stoddard (1965). Nail varnish was used to obtain imprints from the abaxial surface of leaves. The calculation of stomatal density was done from the nail varnish peels which were placed on a haemocytometer slide and viewed through a compound microscope (Model Leitz SM-LUX).

Leaf photosynthesis rate, stomatal conductance and internal CO_2 concentration were recorded using an infrared gas analyser (LCA2- ADC Hoddesdon, UK). The above measurements were determined when plants were 48 days into the treatments. For diurnal determinations of stomatal resistance, a transit time porometer (MK-3 Delta -T Devices, UK) was used. In one of the studies, the changes in stomatal resistance were followed in sequence with the cumulative radiant energy recorded by a solarimeter attached to a microvolt integrator. Measurements were made at two-hour intervals.

RESULTS

Stem Diameter and Plant Height Increment

There was a significant reduction ($P<0.05$) in stem diameter after the plants had been exposed to a cyclical stress regime of more than 6 days *(Fig. 1* and Table 2). After 12 and 24 days of cyclical stress, stem diameter of stressed plants was 50% and 70% lower respectively, compared to the non-stressed plants in the control and to plants subjected to 3 days of water stress (Table 2).

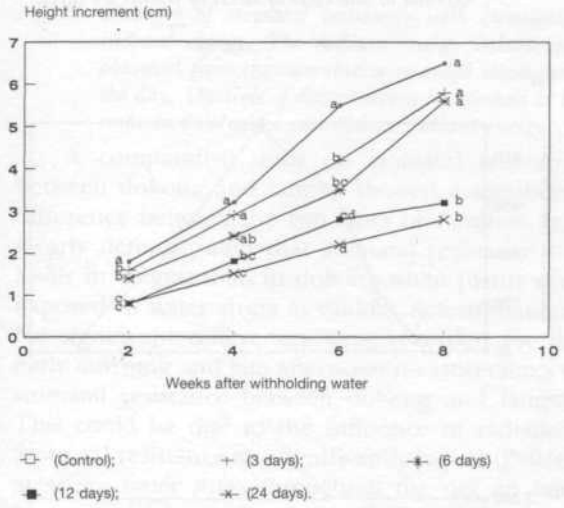

Fig. 1. Height increment as influenced by duration of water stress.

TABLE 2
Effects of water stress on leaf area, stem diameter, leaf elongation and root volume

Treatment (day)	Leaf area (cm^2)	Stem diameter (cm)	Leaf elongation (cm)	Root volume (cm^3)
Control	2111.0a	0.47a	18.73a	33.5a
3	1870.0b	0.39ab	18.20b	32.5a
6	1276.0c	0.37b	15.97c	25.5b
12	937.5d	0.27c	12.97d	18.5c
24	430.0e	0.09d	10.70e	13.0c

Mean separation by DMRT at 5% level

Leaf Area, Leaf Elongation and Root Volume

Leaf growth reduced significantly ($P<0.05$) with increasing water stress. There was a 11%, 40%, 55% and 80% reduction in leaf area with increasing stress for 3, 6, 12 and 24 days respectively. Similar reductions were observed in leaf elongation. Leaf elongation rate for control plants averaged 0.26 cm/day compared to only 0.14 cm/day for plants that were subject to cyclical stress for 24 days. Root volume also decreased with water stress after more than 3 days (Table 2).

LAI, Leaf, Stem and Root Dry Weight

Fig. 2 illustrates the dry weight of leaf, stem and root at final harvest. There was a significant reduction in leaf dry weight which related well with the decrease in leaf area as shown in Table 2. Root dry weight also decreased at higher water stress. There was a larger decline in leaf growth than root growth. Stem dry weight only affected plants undergoing cyclical water stress for more than 6 days. The results clearly demonstrated that LAI was significantly smaller at cyclical water stress after more than 3 days. The reduction in leaf size and the promotion of abscission of the leaves contributed to the lower LAI of stressed plants after the sixth week *(Fig. 3)*.

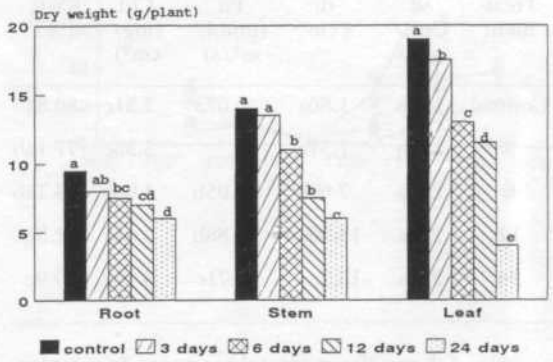

Fig. 2. Dry weight of root, stem and leaf as influenced by duration of water stress.

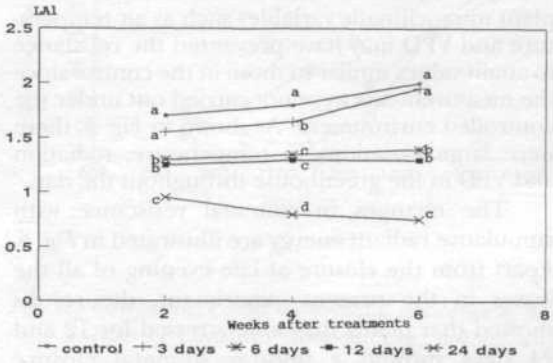

Fig. 3. Leaf area index as influenced by duration of water stress.

Relative Water Content, Stomatal Density, Stomatal Resistance, Chlorophyll Content and Photosynthesis Rate

Relative water content decreased with increasing water stress. At 12 and 24 days of water stress, relative water content was reduced by 8 and 30%, indicating a water deficiency in the leaves. Other stress treatments gave smaller decreases. The results also showed that stomatal density and chlorophyll content per unit area significantly increased with water stress. Increase in stomatal number, however, did not improve stomatal response as its resistance was greater than non-stressed controls in plants. This resulted in a significant fall ($P<0.05$) in photosynthesis rate to 2.05, 1.88 and 0.71 µmol $CO_2/m^2/s$ at 6, 12 and 24 days, respectively (Table 3).

TABLE 3
Stomatal density (sd), diffusive resistance (dr), photosynthesis rate (Pr), chlorophyll content (Chl) and relative water content (RWC) as influenced by water availability.

Treatment	sd Unit/ mm^2	dr s cm^{-1}	Pn (µmol/ m^2/s)	Chl (mg/ cm^2)	RWC (%)
Control	160b	1.86a	6.07a	3.31c	80.8a
3	173b	1.31a	–	3.35c	77.1ab
6	204a	7.69b	2.05b	3.60b	73.7ab
12	204a	13.50c	1.88b	3.64a	72.3b
24	218a	13.7c	0.71c	3.80a	50.9c

Fig. 4 shows the stomatal response to rewatering. In general, stomatal resistance of severely stressed plants did not revert to the control values when water was made available. For moderately stressed plants, the stomatal response indicates a recovery from water stress. The changes in the plant microclimatic variables such as air temperature and VPD may have prevented the resistance to attain values similar to those in the control since the measurements were not carried out under the controlled environment. As shown in *Fig. 5*, there were large variations in temperature, radiation and VPD in the greenhouse throughout the day.

The changes in stomatal resistance with cumulative radiant energy are illustrated in *Fig. 6*. Apart from the closure at late evening of all the leaves in the present experiment, the results showed that plants that were stressed for 12 and 24 days showed a midday stomatal closure approaching 17.2 and 32 sec cm^{-1} respectively. This could be attributed to the high VPD in the plant canopy.

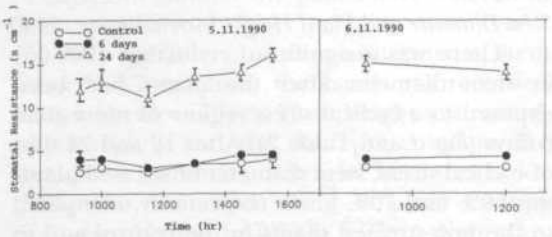

Fig. 4. Response of stomata upon rewatering. Arrows show plants were rewatered. The plants were exposed to one cycle of stress of 6 and 24 days.

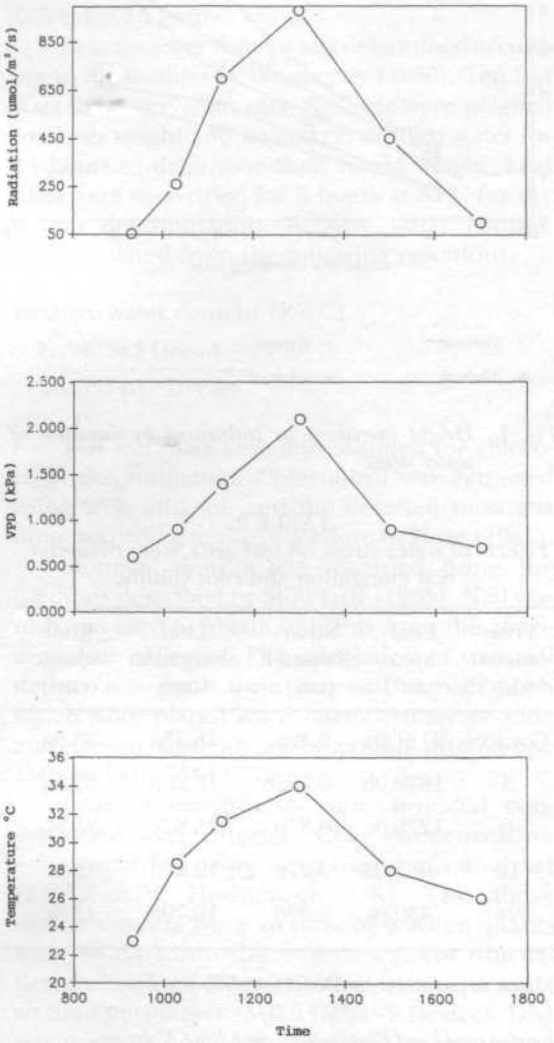

Fig. 5. Variation in air temperature, radiation and vapour pressure deficit (VPD) in the greenhouse

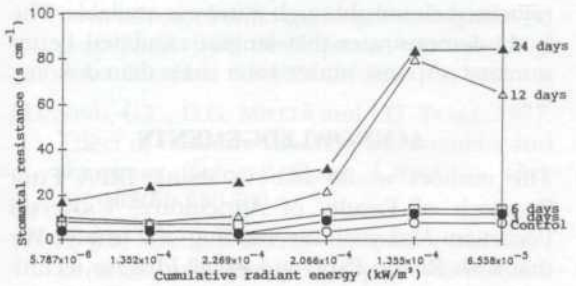

Fig. 6. Changes of stomatal resistance with cumulative radiant energy. The radiant energy values were obtained from the cumulative recorded throughout the day. The time of day is shown in brackets at the respective value for cumulative radiant energy.

A comparative study on stomatal resistance between dokong and langsat showed a significant difference between the two types of *Lansium*. It is clearly demonstrated that stomatal resistance was lower in langsat than in dokong when plants were exposed to water stress at midday determinations. No significant differences were recorded for the early morning and late afternoon measurements of stomatal resistance between dokong and langsat. This could be due to the influence of radiation. Stomatal resistance was significantly higher ($P<0.05$) at severe water stress throughout the day on both langsat and dokong plants *(Fig. 7)*.

DISCUSSION

The present study shows that plant vegetative growth was sensitive to water availability. A small depletion in water content in the leaves as a result of the reduced water availability significantly decreased leaf growth. The reduction in leaf growth has been attributed to a disruption in cell expansion and elongation (Acevedo *et al.* 1971; Hsiao 1973; Bradford and Hsiao 1982). The smaller leaf area as well as reduced leaf photosynthesis per unit leaf area may have contributed to the lower accumulation in dry weight of leaves. The fall in LAI *(Fig. 3)* associated with water stress would indicate that there was a reduction in the radiation interception by the leaves, which could have resulted in the lower accumulation of dry weight by plants. Masri and Boote (1988) reported that LAI of maize and soyabean decreased with drought which caused a similar reduction in the accumulation of dry weight by plants. The other possible reason contributing to the lower LAI is that more leaves are shed under water stress. The inhibition of shoot development under water stress was also reported by Levy *et al*.(1978).

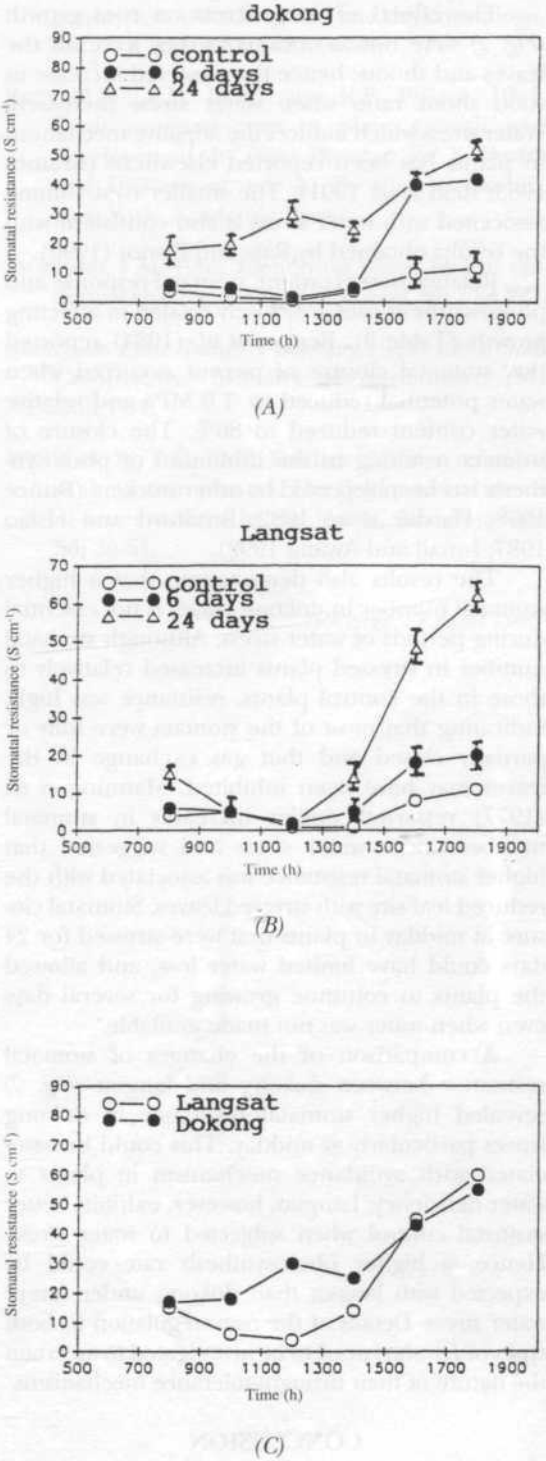

Fig. 7. Comparative data on stomatal resistance of two types of Lansium domesticum, *dokong and langsat. a = dokong, b = langsat, c = comparison of stomatal resistance at severe water stress.*

The effects of water stress on root growth *(Fig. 2)* were not as obvious as they were on the leaves and shoots; hence the observed increase in root: shoot ratio when water stress increased. Water stress which induces the adpative mechanism in plants has been reported elsewhere (Kramer 1983; Reid *et al.* 1991). The smaller root volume associated with water stress is also consistent with the results obtained by Raja and Bisnoi (1990).

Relative water content, stomatal response and photosynthesis rate are closely related in affecting growth (Table 3). Bennet *et al.* (1984) reported that stomatal closure of peanut occurred when water potential reduced to -1.6 MPa and relative water content reduced to 86%. The closure of stomata resulting in the inhibition of photosynthesis has been reported by other workers (Bunce 1978; Harder *et al.* 1982; Bradford and Hsiao 1987; Ismail and Awang 1992).

The results also demonstrate that a higher stomatal number in dokong leaves is not essential during periods of water stress. Although stomatal number in stressed plants increased relatively to those in the control plants, resistance was high, indicating that most of the stomata were fully or partially closed and that gas exchange in the leaves may have been inhibited. Manning *et al.* (1977) reported similar increases in stomatal number under water stress and suggested that higher stomatal resistance was associated with the reduced leaf size with stressed leaves. Stomatal closure at midday in plants that were stressed for 24 days could have limited water loss, and allowed the plants to continue growing for several days even when water was not made available.

A comparison of the changes of stomatal resistance between dokong and langsat *(Fig. 7)* revealed higher stomatal resistance in dokong leaves particularly at midday. This could be associated with avoidance mechanism in plants to water deficiency. Langsat, however, exhibits better stomatal control when subjected to water stress. Hence, a higher photosynthesis rate could be expected with langsat than dokong under severe water stress. Details of the osmoregulation in both types of *Lansium* need to be investigated to ascertain the nature of their drought-tolerance mechanisms.

CONCLUSION

Our studies reveal that reduced water availability inhibited growth and physiological processes of 5-month-old *Lansium* plants. The inhibitions are associated with the reduction in plant water status caused by the depletion of soil water. Recovery of stomatal response was influenced by the intensity of water stress; under severe water stress, stomata remained closed although water was available. The study demonstrates that langsat exhibited better stomatal response under water stress than dokong.

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Morphological Changes with Growth in *Liza carinata* (Valenciennes) Egg, Larva and Juvenile as Distinguished from Those of *Liza haematocheila* (Schlegel)

ANUAR HASSAN[1] and TORU TAKITA[2]
[1]*Faculty of Fisheries & Marine Science, Universiti Pertanian Malaysia, Mengabang Telipot, 21030 Kuala Terengganu, Terengganu, Malaysia.*
[2]*Faculty of Fisheries, Nagasaki University, 1-14 Bunkyo Machi, Nagasaki 852, Japan.*

ABSTRAK

Pertukaran secara morfologi di dalam peringkat telur, larva dan juvena Liza carinata *dengan tumbesaran telah dikaji dalam spesimen yang diternak dan yang ditangkap secara semulajadi.* L. carinata *boleh dibezakan daripada* L. haematocheila *(yang mana mereka bertelur serentak dalam masa dan ruang) dalam peringkat telur, larva dan juvena. Telur menetas dalam masa 45-102 jam selepas penetasan di dalam suhu air 15-21°C, sepadan dengan suhu di tempat peneluran. Larva yang baru menetas mempunyai purata jumlah panjang 2.1 mm. Sirik kaudal "anlage" muncul dalam larva yang mempunyai jumlah panjang 3.4 mm dan peringkat juvena dicapai pada jumlah panjang 8.9 mm.* L. haematocheila *telah dibezakan saiznya yang besar dalam peringkat telur dan larva. Melanofor terletak di atas hujung kepala dan hujung ekor dalam* L. carinata *mungkin juga bertindak sebagai tanda yang boleh membezakan dalam peringkat larva. Unjuran yang lebih panjang di dalam barisan melanofor sisi tengah dalam* L. carinata *membezakan mereka dalam peringkat juvena. Sifat bonjolan dorsal dalam* L. carinata *muncul selepas mencapai jumlah panjang 30 mm, yang mana tidak berlaku dalam* L. haematocheila.

ABSTRACT

The morphological changes in the egg, larval and juvenile stages of Liza carinata *with growth were investigated in reared and wild specimens.* L. carinata *could be distinguished from* L. haematocheila *which is likely to spawn concurrently in time and space in the egg, larva, and juvenile stages. The eggs hatch within 45-102 h after fertilization in water of 15-21 °C, corresponding to the temperature in the spawning ground. A newly-hatched larva averages 2.1 mm in total length. The anlage of the caudal fin appears in the larva of around 3.4 mm and the juvenile stage is attained at a length of 8.9 mm.* L. haematocheila *is distinguished by its larger size in the egg and larval stages. The melanophores located on the top of the head and the rear end of the tail in* L. carinata *may also serve as distinguishing marks in the larval stages. The longer extension of the mid-lateral melanophore row in* L. carinata *distinguishes them in the juvenile stage. The characteristic dorsal ridge in* L. carinata *(which does not occur in* L. haematocheila*) appears after a total length of 30 mm is reached.*

Keywords: morphological change, egg, larva, juvenile, melanophore, spawn, distinguishing mark

INTRODUCTION

Liza carinata (Valenciennes) is a small mullet spread extensively from Japan, Korea, China, and the Malay Archipelago to India and the Red Sea (Fowler 1935; Day 1958; Yoshino and Seno 1984). In Japan, the fish are mainly found along the southern coasts of West Kyushu, but are not commercially important and are treated as garbage fish.

In Ariake Sound, this species occurs with another mullet of the same genus, *L. haematocheila,* which is one of the most commercially important fish in the locality. These two species are likely to spawn concurrently in time and space, at least in a part of the Sound. To investigate the life of the two species it is necessary to distinguish them at early stages of development.

The egg and early stages of *L. haematocheila* have been described in detail (Fujita 1979). However, only a brief description of the morphology of postlarval and juvenile stages of *L. carinata* has been given (Kinoshita 1988), and no effort has been made to distinguish the morphological characters between the two species. Eggs and larvae of both species were obtained by artificial fertilization and larval net surveys in Ariake

Sound (32° 27′ - 33° 11′ N ; 130° 06′ - 130° 37′ E) and neighbouring Chijiwa Bay. Morphological changes with growth in *L. carinata* egg, larva and the juvenile stages as distinguished from *L. haematocheila* were investigated.

MATERIALS AND METHODS

Artificial fertilization of eggs of *L. carinata* was carried out in Chijiwa Bay on April 14, 1988 and that of *L. haematocheila* in Ariake Sound on April 23 and May 5 of the same year. The water temperature of both spawning grounds was around 18°C. The eggs were immediately brought back to the Aquaculture Laboratory of Nagasaki Prefectural Institute of Fisheries and put in water tanks within 2 h 10 min after fertilization. The water temperature of the tanks was then adjusted to ensure that no damage was caused to the embryos by an abrupt change of temperature. Thus the eggs were incubated in water temperatures encountered in the natural spawning ground during the spawning season. The eggs were incubated and the larvae reared in tanks under conditions of ambient room temperature (16-21°C) in the Aquaculture Laboratory. The larvae and juveniles were fed with rotifers, *Brachionus plicatilis* and *Artemia salina* nauplii. Morphological characteristics in individuals raised under artificial conditions and anaesthetized with MS 222 were observed and recorded. The specific differences in morphological characteristics were then confirmed by comparing them with those found in the natural specimens collected in larval nets from the seas in April and May of 1987 and 1988.

RESULTS AND DISCUSSION

Eggs and Embryonic Development

L. carinata eggs are buoyant, nonadhesive, spherical in shape and have a narrow perivitelline space and an extraordinarily large oil globule. These characteristics have been described for mugilid eggs in general (Vialli 1937; Martin and Drewry 1978; de Sylva 1984). *L. carinata* eggs are so buoyant in early stages that the eggs are partially exposed to the air. The eggs gradually become heavier towards the hatching period and sink to the bottom. Such changes in egg buoyancy are known to occur in many fish species (Tanaka 1990). In some mugilids, the eggs are known to sink in late developmental stages, as in *Mugil cephalus*, but not always in *M. capito* (Yashouv and Berner-Samsonov 1970).

The stages of embryonic development of *L. carinata* are presented in Table 1 and *Fig. 1*. The embryonic body starts to form when the germ ring has just passed half of the egg diameter, as judged from a lateral view (*Fig. 1D*). The melanophores appear first on the yolk sac around the embryonic body. Just prior to appearance, uncoloured grains appear on the same areas (*Fig. 1F*). Just after their emergence, the melanophores are only sparsely distributed on the yolk sac and are not present on the oil globule. The oil globule becomes thickly covered with the melanophores on its dorsal surface at a later stage *(Fig.1G)*. The xanthophores are located laterally on the embryonic body and the oil globule and scattered widely on the yolk sac.

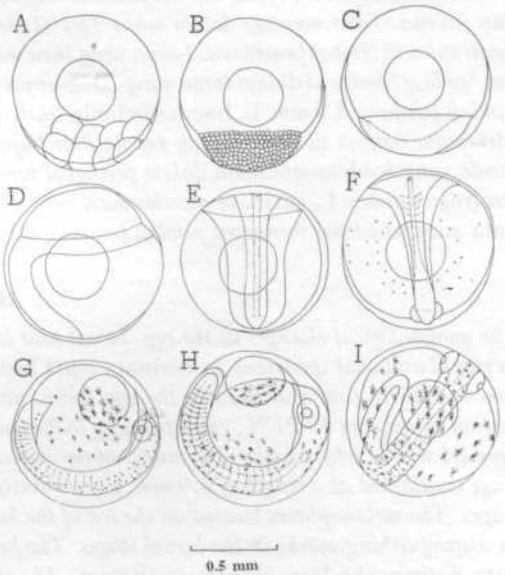

Fig. 1: Embryonic development of L. carinata. *A, eight-cell stage; B, morula stage; C, early gastrula stage; D, formation of embryonic body; E, a little before the closure of the blastopore; F, eye vesicle formation and the appearance of the melanophores; G, tail bud and eye lens formation; H, formation of the tail; I, just before hatching.*

Larvae and Juveniles

The larvae were suspended from mid to lower layers for the first 4 days assuming an upright position with the head down and the tail up. A newly-hatched larva *(Fig. 2A)* averaged 2.1 mm in total length and had 26 (13+13) myomeres. Larvae reach a length of around 2.6 mm in half a day *(Fig. 2B)*, after which growth slows down until the yolk and oil globule are absorbed. The

TABLE 1

Embryonic development of *L. carinata* at various temperatures represented by the times in hours and minutes necessary to reach the respective stages. The eggs were artificially fertilized at 9.50 a.m. on April 11, 1981 and placed in experimental tanks when they were at four-cell stage.

Stage	Incubation temperature (°C)			
	15	17	19	21
Four-cell stage	2:10	2:10	2:10	2:10
Morula stage	5:10	4:40	4:10	3:40
Blastula stage	10:10	8:40	8:10	6:40
Early gastrula stage	18:10	11:40	10:40	9:50
Beginning of embryo formation	26	18	15	14
Closure of blastopore	29	23	18	16
Eye vesicle formation	35	24	19	17
Appearance of melanophores on the yolk sac	37	25	19	17
Kupffer's vesicle formation	39	25	19	17
Appearance of melanophores on the oil globule	40	28	23	20
Appearance of melanophores on the embryo	41	29	24	20
Tail bud formation	42	28	23	21
Appearance of xanthophores	42	30	25	24
Beginning of tail prolongation	45	31	28	26
Disappearance of the Kupffer's vesicle	53	37	31	26
Eye lens formation	51	37	32	28
Beginning of hatching	94	67	53	42
Completion of hatching	102	76	58	45

anus is located slightly posterior of the mid-point of the body until the body begins to grow in height and the anlages of the dorsal and anal fins appear. A large amount of yolk with an oil globule located posteriorly at the bottom of the yolk remains in the newly-hatched larva. The yolk is absorbed within 6 days after hatching, while the oil globule remains for several days more. The day after hatching, black pigments appear on the eyes, and the bud of the pectoral fins emerge. In the three-day-old larva, the mouth is open and the digestive tract is convoluted *(Fig. 2C)*.

The air-bladder is formed within several days after the yolk absorption and the anlage of the caudal fin appears in the larva reaching around 3.4 mm in total length *(Fig. 2E)*. The body noticeably starts to grow in height and the anlages of dorsal and anal fins appear in larva of 4.5 mm. In larva of 4.5 mm *(Fig. 2F)*, the head is round, the body is high, the fin rays of the unpaired fins are under formation, and the pelvic fins are just being formed. In fish reaching around 8.9 mm *(Fig. 2G)*, all the fins are found to be completely formed. The fish reach the juvenile stage before this size, which is slightly smaller than reported by Kinoshita (1988).

Melanophores in a newly-hatched larva are distributed all over the body, dorso-laterally on the yolk sac and dorsally on the oil globule. The xanthophores virtually cover the same locations except in the oil globule, where the melanophores are located dorsally and the xanthophores laterally. As the larva grows, the chromatophores are concentrated in some locations and become sparse on the shoulder and posterior half of the tail. Some melanophores are usually located dorsally and ventrally near the rear end of the tail.

As the larva nears the end of the prolarval stage, the melanophores on the body start to come together on the dorsal and ventral edges and on the mid-lateral line, forming three horizontal lines in lateral view. As the larva grows, melanophores

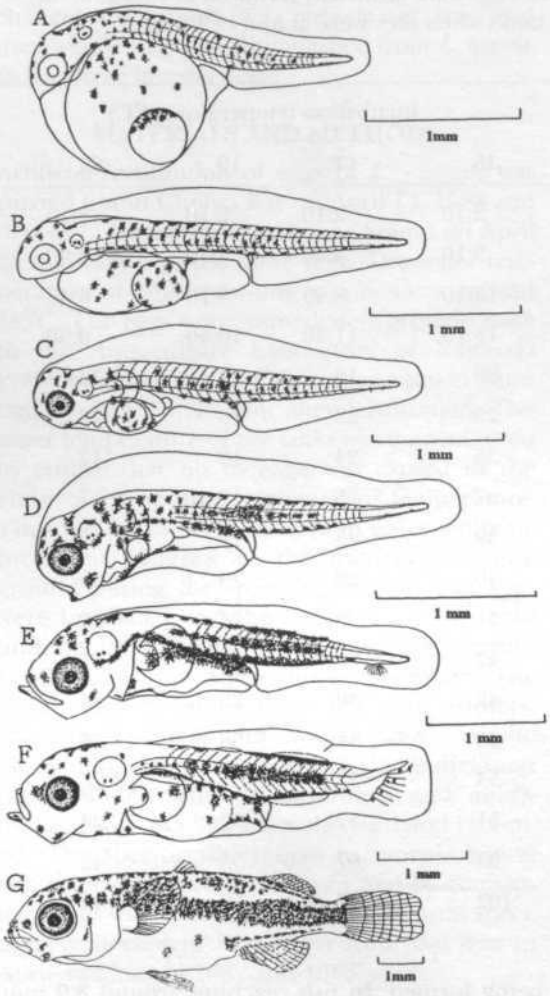

Fig. 2. Morphological development of L. carinata *larvae and juveniles. A, newly hatched, 2.1mm in total length: B, half a day old, 2.6mm; C, 3 days old, 2.9 mm; D, 9 days old. 2.6mm; E, 15 days old, 3.4mm; F, 30 days old, 4.5mm; G, 40 days old, 8.9mm (All drawings and measurements have been made using live specimens).*

covering the body grow thick on the posterior region of the trunk and anterior part of the tail, rendering the myomere uncountable. After the yolk and oil globule are absorbed, the visceral region encasing the air-bladder is covered by thick melanophores. The melanophores are also located on the dorsal surface of the head, the edges of the jaws, the bottom of preopercle and the ventral edge of breast, and these distribution patterns do not change significantly until the larva nears the juvenile stage.

About the time when the caudal fin rays are under formation and the anlages of the dorsal and anal fins appear, the mid-lateral melanophore line starts to broaden, especially on the anterior part of the tail. At the same time, the melanophores on the dorsal sides of the head and body begin to increase.

The guanophores appear sparsely after the postlarva stage is reached and soon become dense all over the body.

Species Differences in the Egg, Larva and Juvenile Stages

The egg and oil globule sizes of artificially fertilized *L. carinata* eggs are shown in Table 2 along with those of *L. haematocheila*. *L. carinata* eggs are smaller in egg size and oil globule size than those of *L. haematocheila*. Although there is an overlap in egg size between the two species, the *L. carinata* egg can be distinguished by the evidently large size of the oil globule, as described by Mito (1988).

The external morphologies of the larval and juvenile stages, including pigmentation are very similar in mugilids, even between genera. Yashouv and Berner-Samsonov (1970) used the presence or absence of chromatophores on the yolk sac, oil globule and forehead as characters which distinguish the mugilids in early larval stages occurring along the Israeli coast. Some descriptions of eggs and larvae of *Liza* species are available (Natarajan and Patnaik 1972; Fujita 1979; Cambray and Bok 1989), but differences are not definite within genera. *L. carinata* has been considered to be difficult to distinguish from *L. haematocheila* in the larval stage (Kinoshita 1988). We studied the reared specimens to determine the distinguishing features, and then confirmed our findings using wild specimens.

L. carinata larvae are apparently smaller than *L. haematocheila* when the stage is defined by morphological features. Five-day-old *L. carinata* prolarvae which have nearly absorbed the yolk but not the oil globule, measure 2.6 to 2.8 mm in notochord length and 2.8 to 3.0 mm in total length after fixation in formalin solution while *L. haematocheila* larvae at the same stage measure 3.0 to 3.2 and 3.1 to 3.5 mm respectively. *L. carinata* postlarvae which have a straight notochord but have yet to reach fin formation measure 2.7 to 3.0 mm and 3.0 to 3.4 mm, while *L. haematocheila* larvae measure 3.4 to 4.0 and 3.7 to 4.2 mm respectively. The *L. carinata* larvae showing caudal fin rays and anlages of the 2nd dorsal and fins measure 3.6 to 4.3 mm and 3.8 to 4.8 mm, while *L. haematocheila* larvae are 4.2 to 5.1 and 4.4 to 5.5 mm respectively.

TABLE 2
Comparison of the egg and oil globule sizes of the two *Liza* species.

		L. carinata	*L. haematocheila*
	x	0.84	0.96
Egg diameter (mm)	Range	0.78-0.93	0.88-1.02
	SD	0.03	0.03
	x	0.32	0.46
Oil globule diameter (mm)	Range	0.27-0.37	0.44-0.49
	SD	0.03	0.01

In prolarva and postlarva stages, the melanophores on the top of the head are distributed irregularly in *L. haematocheila (Fig. 3C and D)*, while in *L. carinata* a comparatively widely branched melanophore is distributed at the posterior end of the cranium *(Fig. 3A and B)*. Up to the stage of caudal fin formation the tiny melanophores located on the rear end of the tail in *L. carinata* may also serve as a distinguishing feature from *L. haemotocheila* larva, which does not have a melanophore in that position.

The criteria used to distinguish the larvae can not be applied to the juveniles, for the melanophores on the head increase in *L. carinata* and their distribution becomes irregular. In *L. carinata* juveniles *(Fig. 3E)*, the mid-lateral melanophore row extends farther ahead than in *L. haematocheila (Fig. 3F)*, although the width of the row and melanophore density vary according to the rearing conditions. The characteristic dorsal ridge in *L. carinata* appears after reaching 30 mm in total length. This ridge does not occur in *L. haematocheila.*

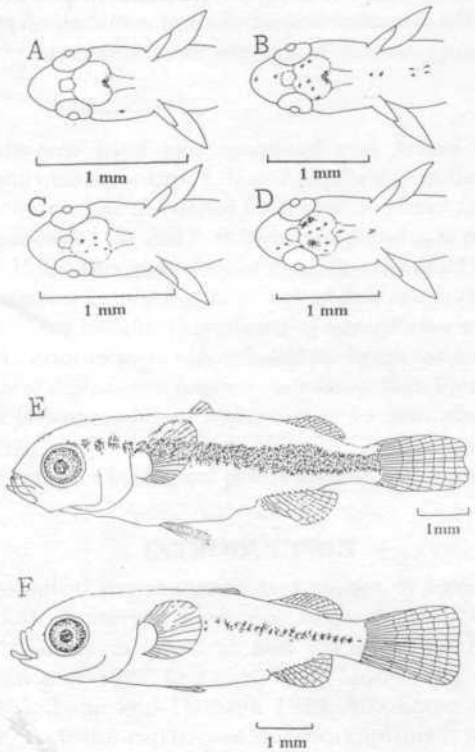

Fig. 3. Comparison of the pigmentation of L. carinata *and* L. haematocheila, *showing the points to distinguish the two species. A to D, anterior dorsal views of the both species; A and B,* L. carinata; *C to D,* L. haematocheila; *A, 2.8 mm total length; B, 4.3 mm; C, 3.4 mm; D, 5.1 mm; E to F, lateral views showing the midlateral melanophore row; E,* L. carinata, *8.9 mm and F,* L. haematocheila, *10.1 mm. (All drawings and measurements have been made using specimens fixed in formalin).*

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Delignification of Palm-press Fibre by White-rot Fungi for Enzymic Saccharification of Cellulose

CHOW-CHIN TONG*, SAW-LEE CHEW and MOHD. NOOR WAHAB
Department of Biochemistry and Microbiology,
Faculty of Science and Environmental Studies
Universiti Pertanian Malaysia.
43400 UPM, Serdang, Selangor Darul Ehsan, Malaysia

ABSTRAK

Hampas kelapa sawit diinokulasi dengan sepuluh jenis kulat rot putih yang berbeza iaitu : Pleurotus sajor-caju *I, II dan III;* Pleurotus florida; Lentinula edodes *I, II, III, IV and V dan* Ganoderma lucidum *sebelum dieramkan selama tiga bulan. Daripada kulat yang dikaji, didapati bahawa* P. sajor-caju *I, III dan* P. florida *merupakan pengurai lignin yang terbaik merendahkan kandungan lignin sebanyak 35% dan meningkatkan kesenangan penghadamam sabut sebanyak 21%. Semasa pertumbuhan kulat tersebut, lignin adalah komponen sabut yang banyak terhapus manakala kehilangannya adalah sedikit bagi selulosa dan hemiselulosa untuk tempoh pengeraman dua bulan. Pemecahan hemiselulosa hanya berlaku selepas penghancuran lignin dan selulosa. Walaupun setengah daripada* L. edodes *yang dikaji menyerang hanya komponen lignin dan biarkan saja komponen selulosa dan hemiselulosa, tetapi kadar pemecahannya lebih rendah berbanding dengan* Pleurotus *spp.* G. lucidum *merupakan pengurai lignin yang lemah dan menggunakan hemiselulosa lebih dari selulosa untuk pertumbuhan.*

ABSTRACT

Palm-press fibres were inoculated with fungal mycelium of ten different isolates of white rot-fungi namely: Pleurotus sajor-caju *I, II and III;* Pleurotus florida; Lentinula edodes *I, II, III, IV and V and* Ganoderma lucidum. *The inoculated fibres were incubated for a period of up to three months. Of the fungi tested,* Pleurotus sajor-caju *I, III and* P. florida *were found to be the best lignin degraders, decreasing the lignin content by as much as 35%. This corresponded to an increase of 21% in the digestibility of the fibres. Lignin showed the largest proportionate loss during the growth of these fungi; cellulose and hemicellulose showed the lowest loss for incubation of up to two months. Degradation of hemicellulose seemed to take place later than lignin and cellulose. Some isolates of* L. edodes *preferably attacked the lignin component while leaving the cellulose and hemicellulose untouched; its rate of degradation however, was slower than* Pleurotus *spp.* G. lucidum *was a poor lignin degrader and under the present conditions preferred to utilise hemicellulose rather than cellulose for growth.*

Keywords : biological pretreatment, palm-press fibre, white-rot fungi

INTRODUCTION

Biological pretreatment techniques of lignocellulosic materials have not been developed as intensively as physical and chemical methods (Fan *et al.* 1982; Lee *et al.* 1983; Moo-young *et al.* 1985; Tong and Hamzah 1989; Adaskaveg *et al.* 1990). If the capacity of microorganisms is to be utilised more fully, a better understanding of microbial lignin degradation is necessary (Crawford 1981).

The most promising organisms for biological pretreatment of lignocellulose are the white-rot fungi (Hatakka 1983). It is possible to use these microorganisms to degrade the lignin component in lignocellulosic waste materials to make the cellulose and hemicellulose components more accessible for further biotechnological use.

Solid state fermentation of wheat straw with white-rot fungi of the genus *Pleurotus* has been shown to increase susceptibility to enzymatic saccharification (Detroy *et al.* 1980; Hatakka 1983; Mueller and Troesch 1986) as well as rumen digestibility (Zadrazil 1977, 1978 and 1980; Lindenfelser *et al.* 1979; McQueen and Reade 1983). Commercial growing of *Pleurotus* is increasing worldwide (Tong and Chen 1990). Thus, simultaneous production of mushrooms and highly digestible material either for enzymatic saccharification or as animal feed would be economically attractive (Bano and Rajaratnam 1982).

In the present work, several mushroom cultures were screened for selective lignin removal.

*To whom all correspondence should be addressed

The aim of this investigation was to use biologically treated palm-press fibres as a substrate in a further biotechnological process, i.e. enzymic sacharification of cellulose for the production of reducing sugars.

MATERIALS AND METHODS

Whatman No. 1 filter paper was obtained from Whatman Ltd. Palm-press fibres were kindly supplied by Sri Langat Palm Oil Mill, Ulu Langat, Selangor. Potato dextrose agar was a product of Difco Laboratories, Detroit USA. Cellulase enzyme from *Trichoderma viride* was purchased from Sigma Chemical Company, St. Louis, USA.

Fungi and Inocula

The following white-rot fungi were obtained from the culture collection of the Department of Biochemistry and Microbiology, Universiti Pertanian Malaysia: *Pleurotus sajor-caju* I, II and III; *Pleurotus florida; Lentinula edodes* I, II, III, IV and V; and *Ganoderma lucidum*. Stock cultures were maintained on potato dextrose agar slants at 4°C. Spawn was prepared from wheat grains. Bottles of sterilised grains were inoculated with actively growing mycelium from potato dextrose agar plates and incubated in the dark at room temperature (28°C) until the mycelium completely covered the grains. Incubation time varied from 16-33 days depending on the species.

Substrate Preparation

Palm-press fibres were soaked overnight in tap water and drained until moderately moist. $CaC0_3$ (3-5%) and rice bran (10%) were added and thoroughly mixed. Approximately 5 g (dry weight) of the fibres were then packed into a 250 ml wide-mouth bottle and autoclaved at 121°C, 15 psi for 1 h. One teaspoon of the spawn was added to the sterilised fibres and incubated in the dark at room temperature for a period of up to three months. At regular intervals of one month, samples were removed and again autoclaved as described above to kill the fungal mycelium. The treated fibres were then dried to a constant weight at 65°C for 24 hours and milled with a blender (National Model MK-C100N) to approximately 2-3 mm in length before analysis for chemical composition. The digestibility test was then carried out on the treated fibres as described below.

Chemical Analysis

The fibre components were estimated individually after a series of extraction procedures. The various components analysed included the lignin, cellulose, hemicellulose, and ash content. The method of Goering and Van Soest (1970) was adopted.

Digestibility Test

The effect of the biological pretreatment was evaluated by comparing the biodegradability of the untreated and treated fibres. Biodegradability of the treated fibres can be measured in an *in vitro* test system by determination of the amount of sugars liberated during incubation with cellulolytic enzymes. The reaction mixtures contained 25 mg fibres, 0.9 ml 0.1 M citrate-phosphate buffer, pH 5.0, 0.1 ml of enzyme solution of appropriate dilution and one drop (10µl) of toluene. After incubation at 37°C for 24 hours, 0.5 ml of the reaction mixture was withdrawn and assayed for reducing sugars.

The number of reducing sugar groups created by hydrolysis of the cellulosic substrate were measured spectrophotometrically by using the Nelson-Somogyi procedure (Nelson 1944; Somogyi 1952) as described earlier (Tong and Rajendra 1992).

RESULTS

The chemical composition of the starting untreated palm-press fibres was estimated to be 39.9% cellulose, 28.9% hemicellulose, 20.3% lignin and 3.6% ash content (Tong and Hamzah 1989). The digestibility test of these fibres showed that about 0.2 mg reducing sugars was released from the fibres under the conditions studied.

Palm-press fibres inoculated with the fungal mycelium were analysed for their chemical composition and digestibility at monthly intervals.

Lignin Content

All the microorganisms grew well on this substrate, but degraded it to a different extent *(Fig. 1a)*. *P. sajor-caju* II and *P. florida* were the best lignin degraders among the organisms tested, reducing as much as 27% of the lignin in the fibres by the end of the first month. This was followed by *P. sajor-caju* III, *L. edodes* II and III. All other organisms were unsuitable for biological delignification at this stage.

After two months of incubation, there was a general decline in the lignin content in all the treatments *(Fig. 1b)*. The rate of delignification was highest in fibres treated with *P. sajor-caju* I where the total amount of lignin removed was comparable to that achieved by *P. sajor-caju* II and III. However, it was *P. florida* that degraded the most lignin.

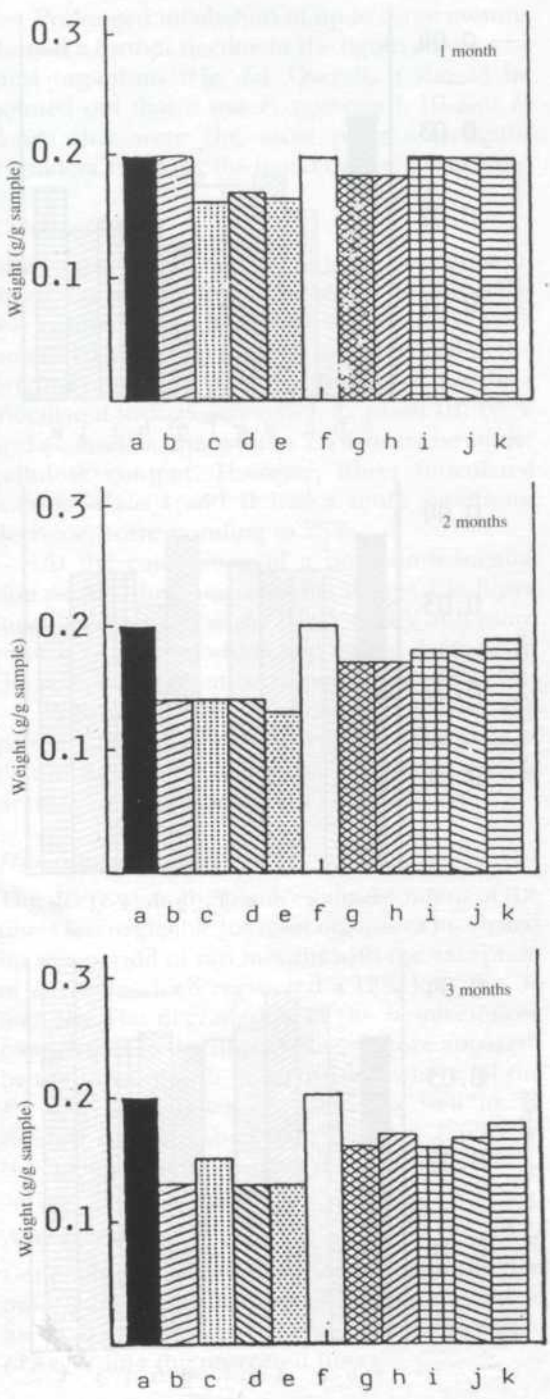

Fig.1: Effect of biological pretreatment by fungi on the lignin content of palm-press fibres. Results represent average of duplicates. The fungi tested were:

(a) *untreated fibres* *(b)* P. sajor-caju *I*
(c) P. sajor-caju *II* *(d)* P. sajor-caju *III*
(e) P. florida *(f)* L. edodes *I*
(g) L. edodes *II* *(h)* L. edodes *III*
(i) L. edodes *IV* *(j)* L. edodes *V*
(k) G. lucidum

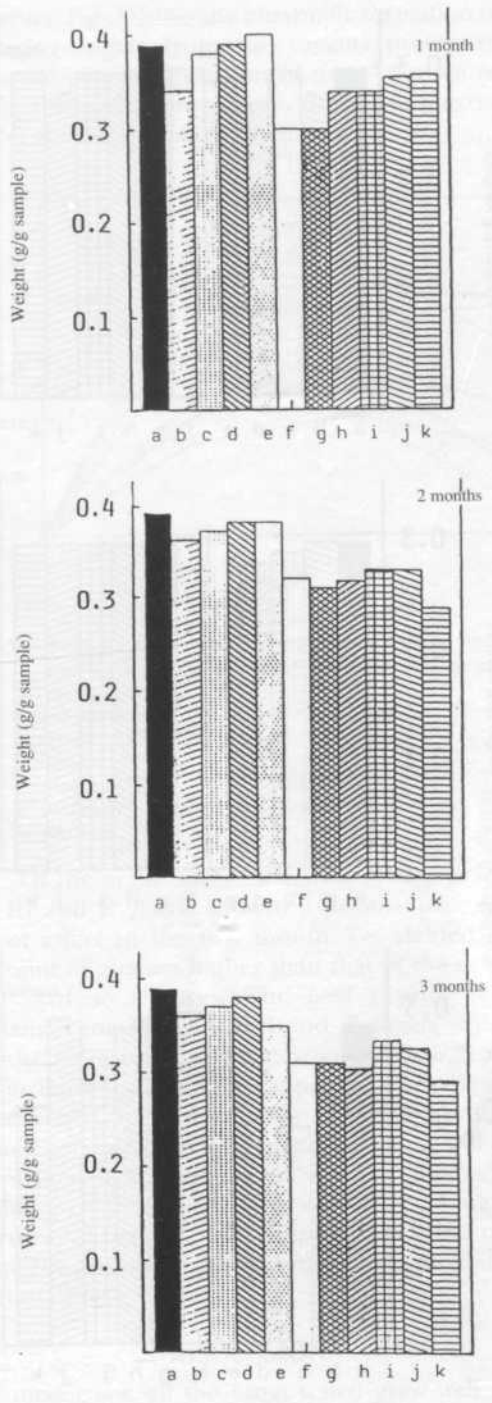

Fig.2: Effect of biological pretreatment by fungi on the cellulose content of palm-press fibres. Results represent average of duplicates. The fungi tested were:

(a) *untreated fibres* *(b)* P. sajor-caju *I*
(c) P. sajor-caju *II* *(d)* P. sajor-caju *III*
(e) P. florida *(f)* L. edodes *I*
(g) L. edodes *II* *(h)* L. edodes *III*
(i) L. edodes *IV* *(j)* L. edodes *V*
(k) G. lucidum

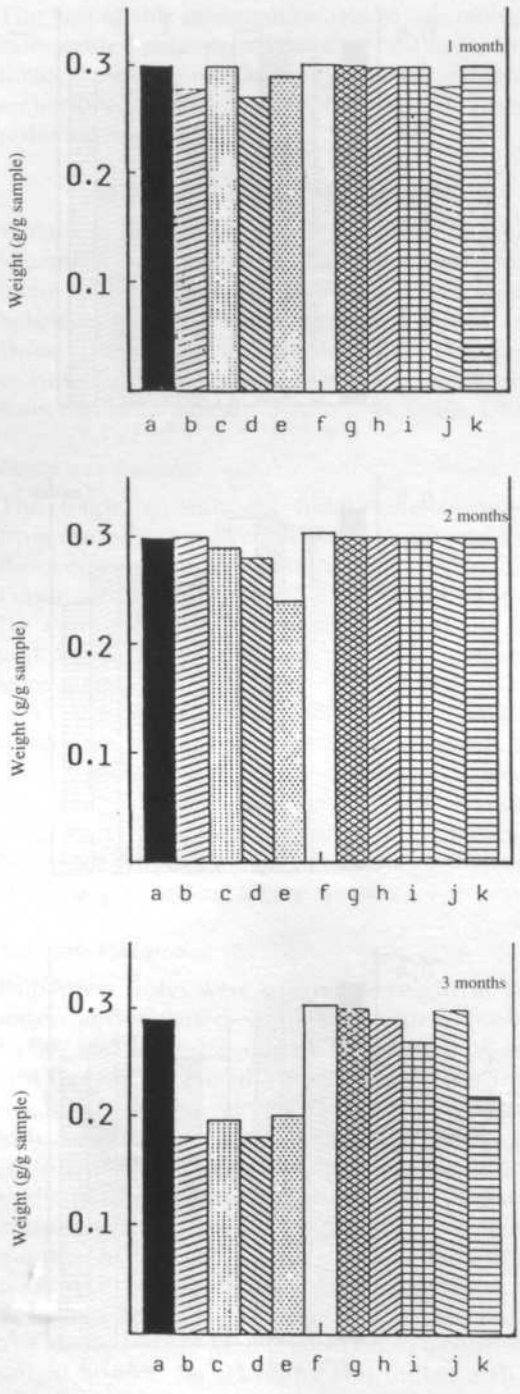

Fig. 3: Effect of biological pretreatment by fungi on the hemicellulose content of palm-press fibres. Results represent average of duplicates. The fungi tested were:

(a)	*untreated fibres*	*(b)*	P. sajor-caju *I*
(c)	P. sajor-caju *II*	*(d)*	P. sajor-caju *III*
(e)	P. florida	*(f)*	L. edodes *I*
(g)	L. edodes *II*	*(h)*	L. edodes *III*
(i)	L. edodes *IV*	*(j)*	L. edodes *V*
(k)	G. lucidum		

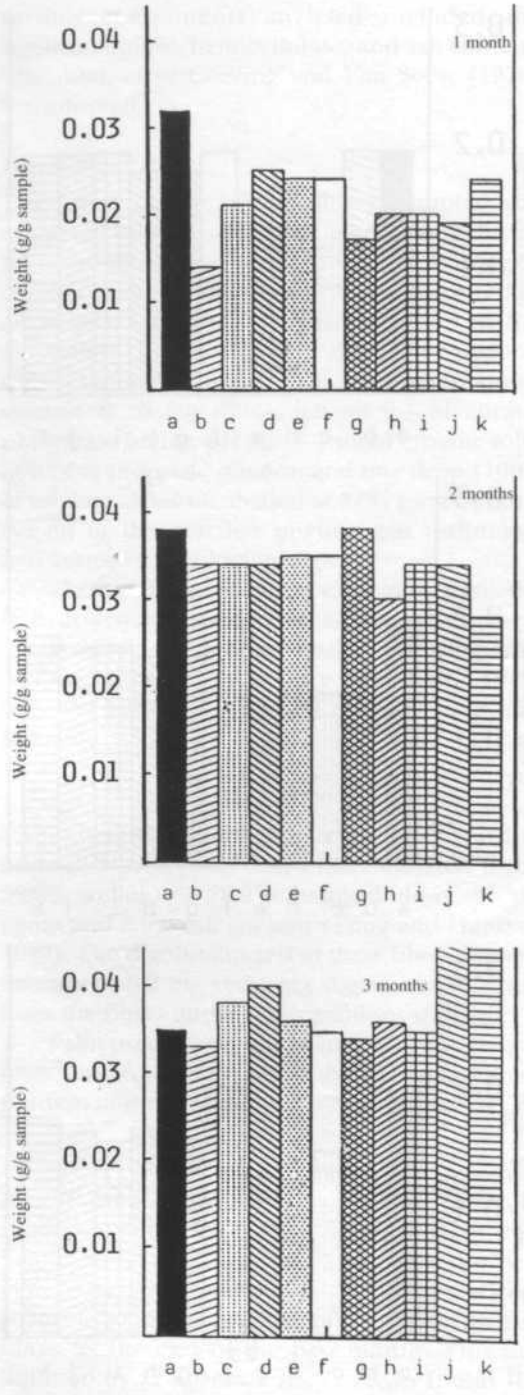

Fig. 4: Effect of biological pretreatment by fungi on the ash content of palm-press fibres. Results represent average of duplicates. The fungi tested were:

(a)	*untreated fibres*	*(b)*	P. sajor-caju *I*
(c)	P. sajor-caju *II*	*(d)*	P. sajor-caju *III*
(e)	P. florida	*(f)*	L. edodes *I*
(g)	L. edodes *II*	*(h)*	L. edodes *III*
(i)	L. edodes *IV*	*(j)*	L. edodes *V*
(k)	G. lucidum		

Prolonged incubation of up to three months showed a further decline in the lignin content by most organisms *(Fig. 1c)*. Overall, it should be pointed out that it was *P. sajor-caju* I, III and *P. florida* that were the most promising lignin degraders, reducing the lignin by as much as 35%.

Cellulose Content

After one month of biological pretreatment, there was no significant difference in the cellulose content of the fibres inoculated with *P. sajor-caju* II, III and *P. florida (Fig. 2a)* compared to the original untreated fibres. In the case of the fibres inoculated with *P. sajor-caju* I, *L. edodes* III, IV, V and *G. lucidum*, there was a 7.6% decrease in the cellulose content. However, fibres inoculated with *L. edodes* I and II had a more significant decrease, corresponding to 25%.

At the completion of a two-month incubation period, there was a further decrease in fibres inoculated with *L. edodes* III, IV and V and more so with *G. lucidum* which had a total decrease in the cellulose content corresponding to 28%.

With a three-month incubation period, the pattern of cellulose content remained the same as that of the second month but registered a slight decrease in fibres inoculated with *P. florida*.

Hemicellulose Content

The decrease in the hemicellulose content of the fibres was negligible for most organisms incubated up to a period of two months with the exception of *P. florida* which registered a 12% loss *(Fig. 3a and 3b)*. The degradation of the hemicellulose component in the fibres became more apparent by the third month of incubation where all the *P. sajor-caju* isolates, *P. florida* as well as *G. lucidum* caused substantial decrease (30%) in the hemicellulose content *(Fig. 3c)*.

Ash Content

Generally, the ash content decreased in the first month *(Fig. 4)* of incubation but continued to increase with prolonged incubation to the extent of exceeding the untreated fibres.

Digestibility Test

With regard to the use of lignocellulosic waste for bioconversion purposes, the enzymatic hydrolysis of cellulose was the most important limiting step (Mueller and Troesch 1986). Biodegradability of the treated fibres was determined *in vitro* by measuring the amount of glucose liberated during incubation with cellulolytic enzymes. *Fig. 5* shows the enzymatic formation of reducing sugars from the various pretreated fibre samples as a function of time. Untreated fibres released approximately 0.2 mg of glucose under the conditions studied.

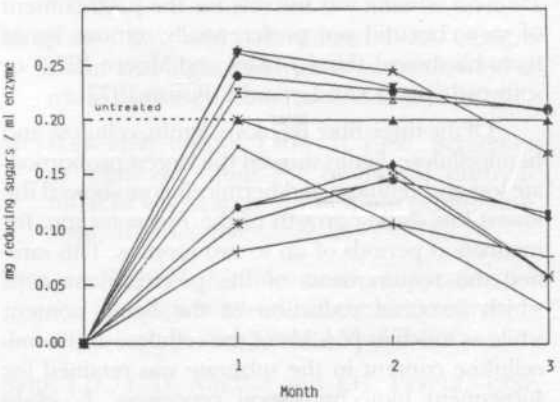

Fig. 5. Reducing sugars released from palm-press fibres treated with different organisms. Each point represents the average of triplicates.

(a)	*untreated fibres*	*(b)*	P. sajor-caju *I*
(c)	P. sajor-caju *II*	*(d)*	P. sajor-caju *III*
(e)	P. florida	*(f)*	L. edodes *I*
(g)	L. edodes *II*	*(h)*	L. edodes *III*
(i)	L. edodes *IV*	*(j)*	L. edodes *V*
(k)	G. lucidum		

Of the organisms tested, only *P. sajor-caju* I, II, III and *P. florida* showed a positive pretreatment effect in the first month, i.e. yielded an amount of glucose higher than that of the original untreated fibres. The best results were obtained for *P. sajor-caju* I and *P. florida* which yielded reducing sugars which amounted to 0.26 mg, an increase of about 30% compared to the control. The actual amount of sugars liberated would have been much higher for reasons which will be explained in the discussion section. Biodegradability of the fibres dropped with prolonged incubation periods. All other organisms did not have the promotary effect on the biodegradability of the fibres.

DISCUSSION

In most cases, all the fungi tested grew well on palm-press fibres, but they increased the digestibility of this substrate to different extents. All components of the lignocellulose could be degraded by white-rot fungi but only those fungi which degraded lignin preferentially can be used for biological pretreatment. Although most of the organisms tested degraded lignin it was *P. sajor-caju* I, III and *P. florida* that were the most promising

lignin degraders, reducing the lignin by as much as 35%. Mueller and Troesch (1986), working with wheat straw treated with *P. florida*, reported a decrease of only 4% in the lignin content. This is understandable because selectivity of attack may depend on the type of lignocellulose materials. *Pleurotus ostreatus* was suitable for the pretreatment of straw but did not preferentially remove lignin from hardwood (birch) (Kirk and Moore 1972) or softword (pine) (Ander and Eriksson 1977).

Of the three fibre fractions, lignin, cellulose and hemicellulose, lignin showed the largest proportionate loss and cellulose and hermicellulose showed the lowest loss during growth of the *Pleaurotus* spp. for incubation periods of up to two months. This satisfied the requirements of the pretreatment steps which favoured reduction of the lignin content while as much as possible of the cellulose and hemicellulose content in the substrate was retained for subsequent biotechnological processess. *L. edodes* (with the exception of *L. edodes* I) seemed to attack lignin preferably while leaving the hemicellulose almost completely untouched but degraded a small amount of the cellulose. Unfortunately, its rate of lignin degradation was slower than the *Pleurotus* spp. Degradation of hemicellulose took place later than cellulose and lignin. *G. lucidum* was a poor lignin degrader and preferred to utilise hemicellulose rather than cellulose for growth.

None of the basidiomycetes tested was able to remove only the lignin component in lignocellulosic material throughout the entire three-month incubation period. Nevertheless, some fungi degraded lignin preferentially over the cellulose and hemicellulose component within the first two months. *P. sajor-caju* I, III and *P. florida* would be ideal for biological pretreatment of fibres because of their higher affinity for lignin.

Good delignification is not in all cases equivalent to good digestibility. Therefore, a digestibility test with cellulases is important in estimating the usefulness of a fungus for biological pretreatment of lignocellulosics. Of the organisms tested, it was only *P. sajor-caju* I, II, III and *P. florida* that showed increase in digestibility of the fibres after biological pretreatment. However, the cellulase digestibility of the fibres declined somewhat after the first month although the lignin content continued to drop. Similarly, all other organisms seemed to have a negative pretreatment effect even though delignification took place. Most probably a new physical barrier was built up by the thick growth of the fungal mycelium covering the fibres which hindered the hydrolysis effect of the cellulase enzymes. Thus the sugar yields following enzymic hydrolysis were lower in this case where washing to remove the mycelial barrier was not carried out. Such a decrease in cellulase digestibility was also reported by Tsang *et al.* (1987). In addition, water-soluble lignin degradation products may have repressed the action of cellulolytic and hemicellulolytic enzymes (Reid *et al.* 1982; Hatakka 1983).

Cultivated mushrooms are normally harvested over a period of several months in the farm. Based on the present results, such a lengthy growth period would presumably have the advantage of increasing the total amount of lignin lost since lignin content continued to decrease with prolonged incubation. However, its effect on the cellulose and hemicellulose components of the fibres would have to be ascertained under mushroom-growing conditions.

To help minimise the cost of any biotechnological process that utilises biological pretreatment, it may be feasible to couple the needs of that process with the methodology and by-products of another industry such as the mushroom industry. Many exotic mushrooms are now produced commercially on supplemented sawdust or palm-press fibres in Malaysia (Tong and Chen 1990). Having harvested the mushrooms, the spent substrate, which was normally considered as waste by the mushroom industry, could then be utilised for subsequent biotechnological processes. In addition, the luxurious growth of the fungal mycelium in the spent substrate could serve as a rich protein source for animal feed. Thus, the simultaneous production of mushrooms and a highly digestible substrate for further biotechnological processess would be economically attractive.

Rapid progress in lignin biodegradation (Crawford 1981) resulting from optimization of cultivation conditions (Kirk *et al.* 1978) has, in recent years, made it possible to accelerate lignin degradation in natural substrates. Therefore, it may be possible in the near future to improve on the selectivity of attack on lignin by white-rot fungi by choosing suitable conditions which stimulate lignin degradation, and which at the same time repress degradation of polysaccharides. This procedure, involving microbial delignification and production of useful products, offers the possibility of utilising and removing the waste palm-press fibres in a completely biological way.

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Maintaining the Colour, Texture and Vitamin C of Cold-stored Pineapples through Shrinkwrapping and Surface-coating with Liquid Paraffin.

SUHAILA MOHAMED and SAFIAH ABDULLAH KHIR
Department of Food Science
Faculty of Food Science and Biotechnology
Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRAK

Pembungkusan mengecut etylen ketumpatan rendah nenas Moris yang disimpan pada 10°C, 15°C, 20°C dan suhu bilik, dapat mengurangkan kehilangan berat dan tekstur buah. Salutan permukaan dengan paraffin menghalang kehitaman yang berlaku dalam buah semasa penstoran pada suhu rendah dan dapat mengekalkan kandongan vitamin C yang tinggi. Salutan paraffin amat berkesan mengekalkan warna nenas segar dan mengurangkan keasidan. Paduan salutan paraffin dan bungkusan-mengecut didapati paling berkesan mengekalkan kesemua parameter yang dikaji. Nenas kawalan yang tidak diberi rawatan mengalami kehitaman isi, kehilangan berat dan penurunan tekstur yang keterlaluan, menjadi terlalu ranum dan diserang kulat semasa penstoran sejuk. Kehilangan berat didapati berhubung secara negative dengan kandongan asid askorbik dan tekstur pulpa dalam kebanyakan keadaan.

ABSTRACT

Low density polyethylene shrinkwrapping significantly reduced weight loss and texture loss in Mauritius pineapples stored at 10°C, 15°C, 20°C and ambient temperatures. Surface coating with paraffin inhibited internal browning in cold-stored pineapples and helped retain a high vitamin C content in the pineapples during storage. Paraffin coating was most effective at maintaining the colour of fresh pineapples and reducing the acidity. A combination of paraffin waxing and shrinkwrapping was found to be effective in maintaining all the parameters studied. Control untreated pineapples exhibited high weight loss, texture loss, over ripening, fungal attack and internal browning during cold storage. Weight loss was found to be negatively correlated to ascorbic acid content and pulp texture under most conditions.

Keywords: paraffin coating, shrinkwrapping, pineapples, 10°C, 15°C, 20°C, weight loss, texture, internal browning, vitamin C, colour

INTRODUCTION

The main problems encountered during storage of pineapples *(Ananas comosus)* are weight loss, fungal attack, and an internal browning (IB) disorder which reduce saleability. Weight loss (resulting in shrivelling and poor external appearance) can be controlled by storage at high relative humidity or by applying surface coatings together with low temperature. Both methods have problems: too low a storage temperature can cause chilling injury (Paull and Rohrbach 1985), and surface coatings can enhance fungal attack and anaerobic off-flavours (Chace and Pantastico 1975). Ripening is delayed by low temperature storage, surface coatings or post-harvest chemical treatments (Wardlaw 1937). Wax or other coatings applied to the surface of fruits influence their physiology and metabolism by limiting gaseous and moisture exchange (Lowings and Cutts 1982). The occurrence of internal browning together with presence of white fruitlets in Mauritius pineapples were reported by Abdullah and Rohaya (1983) for fruits stored at 8 and 12°C followed by one week at 28°C. Waxing pineapples using mineral oil (liquid paraffin) either before or immediately after exposure to chilling temperatures was equally effective in reducing IB symptoms (Paull and Rohrbach 1985).

This paper reports the effects of paraffin waxing and shrinkwrapping on weight loss, texture, colour, total soluble solids (TSS), vitamin C, pH and titratable acidity in Mauritius pineapples stored at various temperatures.

MATERIALS AND METHODS

Pineapples cv Mauritius were bought from MARDI, Klang. Pineapples were selected at the 90% ripe stage (those which have two rows of yellow-coloured eyes), weighed and subjected to various treatment and storage conditions one day after harvest.

Liquid Paraffin Treatment

Fruits were dipped for 30 seconds in 50% aqueous emulsion of liquid paraffin (BDH Limited, Poole, England).

Shrink-wrapped Packaging

Fruits were wrapped with a double layer of low density polyethylene shrinkwrap (0.04 mm thickness) using 'Hot air tunnel' machine model YPS-105.

Groups of 48 fruits were sorted and stored at 10°C, 15°C, 20°C and at ambient temperature (27 ± 3°C). The untreated fruits were used as control.

Assessment of the Stored Fruits

Fruits were assessed for weight loss, pulp colour, texture, sugar, vitamin C, titratable acidity, TSS and pH. Colour was determined using the Hunterlab Tristimulus Colorimeter, expressed as lightness (L), redness (a), and yellowness (b values), with the yellow tile No. C2-22954 as reference (L = 77.5, a = 3.5, b = 23.0). The Instron Universal testing machine with a probe attachment was used for texture measurements on halved fruit. A drive speed of 50 mm/min and a chartspeed of 100 mm/min were used to determine the yield force. A maximum load of 5 kg was used for penetration of the pulp and 20 kg load was used for penetration through the skin. Vitamin C and titratable acidity were determined according to Ranganna (1977). Total soluble solids were determined by using an Otago Hand Refractometer. pH was determined by using Hanna Instruments 8417 pH meter.

Each result is an average of eight readings from four fruits. Fruits were analysed weekly for storage at 10°C, 15°C, and daily for room temperature storage. Samples were picked at random for the analysis.

Sensory evaluation of the pineapples was carried out by 10 trained panellists for colour, taste, texture and flavour on a hedonic scale of 1-7 (1 = dislike very much; 7 = like very much).

Variance analysis and Duncan's multiple range test were applied to all data using SAS statistical software.

RESULTS

Weight Loss

At all temperatures, weight loss increased with time *(Fig. 1)*. Highest weight loss was observed in the control pineapples for all temperatures. Weight loss on paraffin-treated fruits was lower than in control samples. Weight loss by shrinkwrapped pineapples was least.

Fruit Firmness

The graphs for both the skin and pulp firmness showed reduced firmness with increasing storage period, under all storage conditions *(Fig. 2)*. This reduction in firmness was least in shrinkwrapped samples and most in paraffin-treated pineapples.

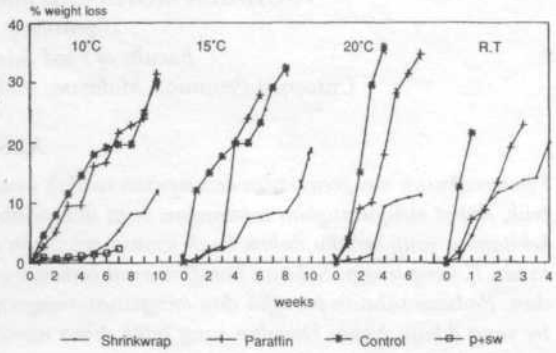

Fig. 1: Weight loss in pineapples stored at 10°C, 15°C, 20°C and room temperature.

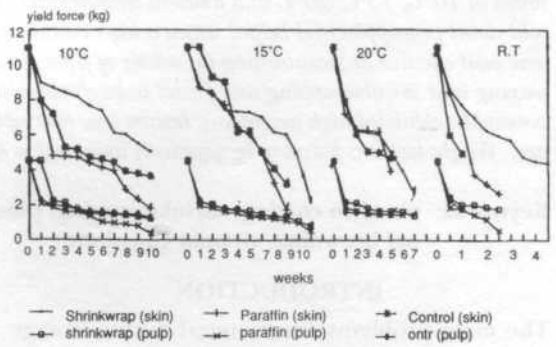

Fig. 2: Texture of pineapples (fruit/pulp) stored at 10°C, 15°C, 20°C and room temperature.

Pulp Colour

After 10 weeks storage at 10°C, paraffin-treated pineapples had the highest lightness (L) values, and shrinkwrapped fruits had the lowest values *(Fig. 3)*. The paraffin wax appeared to slow the rate of ripening of the pineapples, hence delay the change of colour from yellow to orange compared to shrinkwrapped, or control fruits. The pulp of the shrinkwrapped fruits tended to be more orange as can be seen also by the higher (a+b) values than the controlled or paraffin-treated fruits.

Black heart disorder was found most prominent in controlled fruits. The approximate times taken (weeks) for pineapples to develop black heart at the various temperatures of storage and

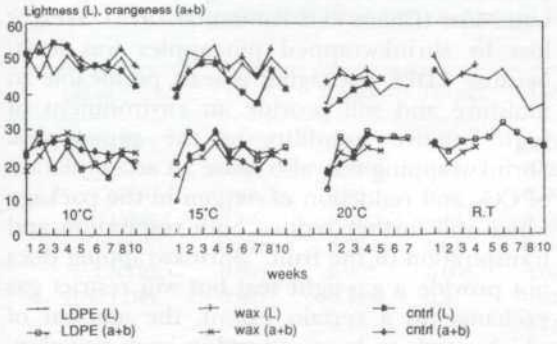

Fig. 3: Colour changes in pineapples stored at 10°C, 15°C, 20°C and room temperature.

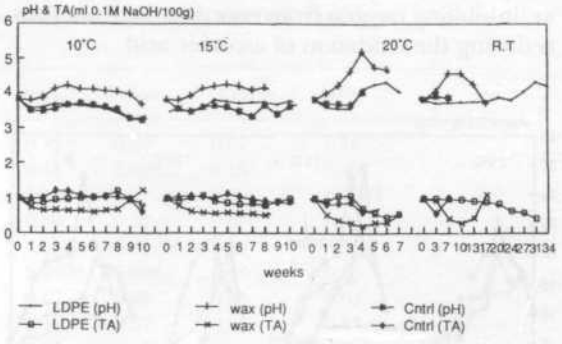

Fig. 4: pH and titratable acidity of pineapples at 10°C, 15°C, 20°C and room temperature.

under the different treatments are listed in Table 1. The appearance of black heart was delayed by waxing and shrinkwrapping.

TABLE 1
The approximate time (weeks) for pineapples to develop internal browning

Temperature	Control	Shrink-wrapped	Paraffin wax
10°C	6	7	11
15°C	3	5	10
20°C	1	3	7

Ambient fruits did not show IB but were unacceptable by the 3rd-4th week

Unlike the control fruits the pineapple skin colour of paraffin-treated fruits remained green and the flesh remained pale yellow showing that ripening was retarded with the paraffin treatment.

pH and Titratable Acidity

Paraffin waxing significantly reduced the acidity of the pineapples on storage for all the temperatures studied. Even shrinkwrapped fruits appeared to have a reduced acidity compared to control fruits but it was not as pronounced as in paraffin-waxed fruits. The degree of acidity developed in the fruit could be related to the availability of oxygen to the fruit. pH was found to be correlated to titratable acidity for pineapples stored at 20°C and room temperature but not for pineapples stored at 10°C and 15°C. pH was also found to be correlated to ripeness or orange colour (a+b values) for paraffin-waxed fruits.

Soluble Solids Content (SSC)

There was a general decrease in SSC content of pineapples on storage except in paraffin-treated and control fruits stored at 10°C and 15°C *(Fig. 5)*. SSC was found to be correlated to ripeness or orange colour (a+b values) for control fruits stored at 15°C and 20°C.

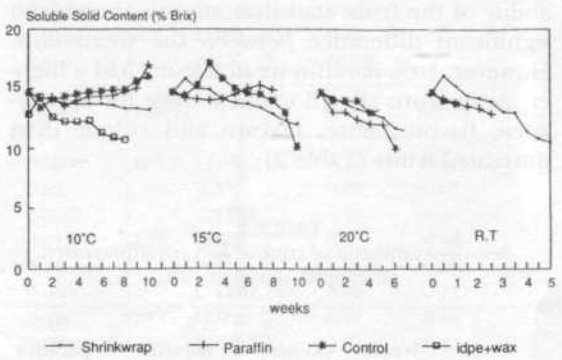

Fig. 5: Total soluble solids in pineapples stored at 10°C, 15°C, 20°C and room temperature.

Ascorbic Acid

There was an initial increase followed by a gradual decrease in ascorbic acid content of pineapples on storage indicating ripening and senescence *(Fig.6)*. Ascorbic acid has been repeatedly shown in various fruits, to increase during ripening and decrease during senescence even on storage (Khin 1991; Ku-Natrah 1992). At the lower temperatures of storage, the paraffin waxed fruits had the highest ascorbic acid content followed by shrinkwrapped fruits showing that paraffin waxing increased the vitamin C content of pineapples

especially those stored at low temperatures. This was probably because paraffin acted as a gas barrier, inhibiting oxygen from entering the fruit, thus reducing the oxidation of ascorbic acid.

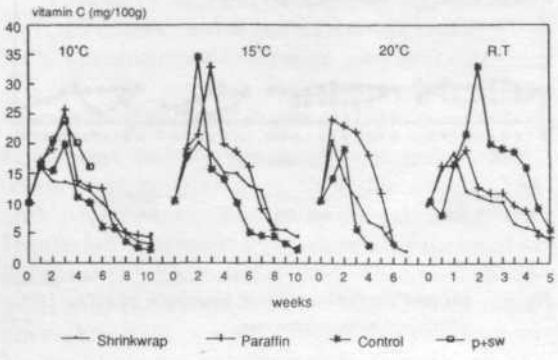

Fig. 6: Vitamin C content in pineapples stored at 10°C, 15°C, 20°C and room temperature.

Sensory Evaluation

Results of the sensory evaluation for control, 5% paraffin and 10% paraffin-treated fruits stored at 10°C are shown in Table 2. Because of the variability of the fruit, statistical analysis showed no significant difference between the treatments. However, 10% paraffin-treated fruits had a higher mean score after 8 weeks storage for appearance, flavour, taste, texture and colour than untreated fruits (Table 2).

TABLE 2
Sensory evaluation of control and paraffin-treated pineapples stored at 10°C

	Week	Control	5% paraffin	10% paraffin
appearance				
	4	a 6.20 A	a 6.80 A	a 5.01 A
	8	b 3.45 A	b 4.00 A	a 4.30 A
flavour				
	4	a 5.45 A	a 5.40 A	a 5.35 A
	8	a 4.35 A	a 5.55 A	a 4.80 A
taste				
	4	a 6.00 A	a 5.55 A	a 5.85 A
	8	a 5.00 A	b 3.00 B	a 6.15 A
texture				
	4	a 6.35 A	a 6.25 A	a 6.15 A
	8	a 5.70 A	b 3.35 B	a 5.75 A
colour				
	4	a 5.80 A	a 6.10 A	a 6.15 A
	8	a 5.50 A	b 4.25 B	a 5.65 A

Values followed by different letters differ significantly
Capital letters show significant difference between treatments
Small letters show significant difference between weeks

DISCUSSION

Paraffin waxes restrict both gas exchange and water loss (Chace and Pantastico 1975). Weight loss by shrinkwrapped pineapples was least, because LDPE packaging is least permeable to moisture and will provide an environment of high relative humidity to the pineapples. Shrinkwrapping may also cause an accumulation of CO_2 and reduction of oxygen in the package which will further reduce both respiration and transpiration of the fruit. Shrinkwrapping does not provide a gas-tight seal but will restrict gas exchange to a certain extent, the amount of which needs to be measured in each situation. This study was not done due to the lack of facilities here. Undoubtedly the main effect is the prevention of water loss.

The pulp firmness of pineapples was found to be correlated with weight/moisture loss (Table 3), accounting for the good firmness reading of shrinkwrapped fruits. As explained previously, high CO_2 atmosphere which is present within the shrinkwrap packaging also inhibits breakdown of pectic substances in other fruits so that a firmer texture is retained for a longer period (Wills *et al.* 1981). It was found that for paraffin waxing the concentration of paraffin used caused lesions to the surface of the pineapples and was detrimental to the surface texture. Work will be carried out to find out the optimum concentration of paraffin to be used for waxing of pineapples in the near future. It was also found that pineapple texture was positively correlated to ascorbic acid content (Table 3).

Paraffin treatment significantly retarded the ripening of pineapples since the skin remained green and the flesh remained pale yellow during storage at 10°C. Paraffin treatment also retarded the development of physiological internal browning symptoms known as 'black heart' which occurs in cold-stored pineapples. This is in agreement with the report by Paull and Rohrbach (1985), on pineapples stored below 21°C. Other surface coatings have been reported to reduce the susceptibility of bananas to chill damage (Lowings and Cutts 1982). Surface coatings have been reported to reduce carotenogenesis in mangoes (Dhalla and Hanson 1988), reduce the rate of chlorophyll breakdown in banana skin (Banks 1984); retard yellowing in plantains (Olorunda and Aworth 1984), and to be more effective and cheaper than gibberellic acid in delaying the degreening of limes (Motlagh and Quantick 1988).

Waxing of fruits and exposing fruit to high temperatures (> 32°C) for a short period (24 h)

TABLE 3
Correlation coefficients (r squared values) of various parameters under different conditions

pH vs	TA	Texskn	Texplp	TSS	Vit C	Wt loss	Lvalue	(a+b)
10°C LDPE	0.125	0.66	0.08	0.43	0.72	0.82	0.07	0.18
WAX	0.03	0.0005	0.02	0.48	0.04	0.028	0.15	0.49
CNTRL	0.03	0.03	0.09	0.26	0.12	0.2	0.09	0.0004
15°C LDPE	0.16	0.31	0.43	0.52	0.39	0.37	0.002	0.07
WAX	0.67	0.29	0.62	0.105	0.012	0.65	0.56	0.5
CNTRL	0.005	0.003	0.008	0.001	0.0009	0.008	0.54	0.16
20°C LDPE	0.92	0.44	0.6	0.62	0.67	0.92	0.01	0.03
WAX	0.88	0.19	0.72	0.21	0.19	0.37	0.36	0.77
CNTRI	0.999	0.15	0.36	0.986	0.37	0.35	0.51	0.6
R.T LDPE	0.777	0.51	0.78	0.84	0.6	0.67	0.58	0.008
WAX	0.9	0.05	0.05	0.15	0.66	0.23	0.89	0.75
CNTRL	0.776	0.54	0.53	0.39	0.1	0.28		

Lvalue vs	Texskn	Texplp	TSS	Vit C	Wtloss	[Asc acid vs	]	Wtloss
10°C LDPE	0.005	0.26	0.0088	0.1	0.04	[10°C	LDPE]	0.92
WAX	0.25	0.18	0.09	0.05	0.27	[	WAX]	0.71
CNTRL	0.28	0.26	0.59	0.76	0.65	[	CNTRL]	0.78
15°C LDPE	0.025	0.0001	0.0008	0.016	0.03	[15°C	LDPE]	0.76
WAX	0.03	0.26	0.03	0.014	0.35	[	WAX]	0.33
CNTRL	0.08		0.35	0.15	0.012	[	CNTRL]	0.57
20°C LDPE	0.039	0.163	0.02	0.003	0.0025	[20°C	LDPE]	0.82
WAX	0.22	0.04	0.61	0.02	0.35	[	WAX]	0.91
CNTRL	0.9		0.47	0.63	0.997	[	CNTRL]	0.68
R.T LDPE	0.07	0.13	0.61	0.38	0.016	[R.T	LDPE]	0.17
WAX	0.34	0.14	0.19	0.11	0.057	[	WAX]	0.002

(a+b) vs	Texskn	Texplp	TSS	Vit C	Wtloss	[TSS vs	]	Vit C	Wtloss
10°C LDPE	0.096	0.3	0.003	0.09	0.0000	[10°C	LDPE]	0.21	0.16
WAX	0.26	0.35	0.09	0.03	0.22	[	WAX]	0.03	0.07
CNTRL	0.02	0.08	0.0009	0.53	0.0036	[	CNTRL]	0.21	0.4
15°C LDPE	0.015	0.02	0.107	0.18	0.013	[15°C	LDPE]	0.51	0.43
WAX	0.12	0.02	0.11	0.07	0.44	[	WAX]	0.0058	0.001
CNTRL	0.14	0.4	0.86	0.0005	0.068	[	CNTRL]	0.02	0.11
20°C LDPE	0.45	0.24	0.18	0.59	0.39	[20°C	LDPE]	0.72	0.69
WAX	0.019	0.04	0.33	0.37	0.0041	[	WAX]	0.6	0.4
CNTRL	0.52	0.15	0.87	0.63	0.82	[	CNTRL]	0.45	0.47
R.T LDPE	0.32		0.0000	0.09	0.15	[R.T	LDPE]	0.48	0.37
WAX	0.34		0.12	0.1	0.004	[	WAX]	0.03	0.64

Texskn vs	Texplp	TSS	Vit C	Wtloss	[Pulp texture vs	]	TSS	Vit C	Wtloss
10°C LDPE	0.04	0.02	0.21	0.29	[10°C	LDPE]	0.02	0.3	0.21
WAX	0.08	0.07	0.79	0.38	[	WAX]	0.07	0.53	0.006
CNTRL	0.002	0.31	0.67	0.27	[	CNTRL]	0.31	0.58	0.61
15°C LDPE	0.62	0.24	0.65	0.89	[15°C	LDPE]	0.24	0.84	0.76
WAX	0.2	0.05	0.75	0.06	[	WAX]	0.05	0.0000	0.82
CNTRL	0.67	0.22	0.49	0.44	[	CNTRL]	0.22	0.06	0.87
20°C LDPE	0.13	0.51	0.77	0.05	[20°C	LDPE]	0.51	0.32	0.57
WAX	0.14	0.03	0.71	0.67	[	WAX]	0.03	0.996	0.4
CNTRL	0.5	0.18	0.43	0.0008	[	CNTRL]	0.18	0.039	0.89
R.T LDPE	0.19	0.04	0.31	0.76	[R.T	LDPE]	0.04	0.0000	0.16
WAX	0.19	0.86		0.49	[	WAX]	0.86		0.91

before or after chilling stress can control storage-induced black heart (Akamine *et al.* 1975). Prolonged storage periods at temperatures less than 12°C have been reported to reduce IB symptom development and the number of affected fruit (Paull and Rohrbach 1985). This was ascribed to damage to the metabolic pathway leading to browning involving monophenols which are ortho-hydroxylated and then oxydised to the resultant catechol derivative, or brown pigments (Singleton 1972). The enzyme tyrosine ammonium lyase responsible in pineapples (Sun 1971) is not hindered by low oxygen concentration. However, the browning reaction was found to require more than 5% oxygen concentration for linking of oxygen with the polyphenol oxidase cuprous ion, and at the same time binding with the aromatic ring converting phenol to phenolate (Bright *et al.* 1963; Singleton 1972). Van Lelyveld and De Bruyn (1977) identified the phenolic compounds as p-coumaric acid, ferulic acid, cafeic acid and sinapic acid. Polyphenol oxidase activity has been shown to increase 30 times in pineapple fruit in response to chilling (Walker 1975). Increased phenolic cinnamic acids have been identified in pineapples with black heart symptoms (Van Lelyveld and De Bruyn 1977). Teisson *et al.* (1979) reported that although a larger volume of fruit was affected at 5°C, the browning was less intense. Rohrbach and Paull (1982) explained the effectiveness of fruit waxing and polyethylene shrinkwrapping by the low oxygen levels after chilling which perhaps reduced black heart development.

Pineapples treated with paraffin seemed to have significantly lower titratable acid values compared to the control. Surface coatings have been reported to differentially alter the permeability of banana skin to CO_2 and O_2, reducing the internal O_2 content without a concomitant rise in CO_2 levels (Banks 1984), and retard pH depression in plantains (Olorunda and Aworth 1984).

The two main factors which affect the soluble solids content of a fruit are:

(a) enzymic breakdown of insoluble polysaccharide (in this case most probably pectic substances) to soluble solids which increase the soluble solids content of the fruit. In some plant products this enzymic reaction is reversible and the direction of reaction is dependent on temperature.

(b) utilisation of the soluble solids (sugars, etc.) for respiration by the fruit which will decrease the TSS content of the fruit.

It appears that at 10°C the respiration of the fruit is considerably lowered; therefore there is very slow reduction is soluble solids. For the control and waxed fruits, the breakdown of insoluble polysaccharide (most likely pectic substances) resulted in a net increase in TSS. For shrinkwrapped fruits the breakdown of polysaccharide was probably also reduced (as verified by the good maintenance of texture of shrinkwrapped fruits at 10°C *(Fig. 2)* contributing to a net reduction of TSS content at that low temperature.

Correlation Studies

The ascorbic acid contents were correlated to the pineapple pulp colour (L and a+b values) and soluble solids content (SSC) only in the control samples (Table 3). Ascorbic acid levels have been associated with the degree of blackheart symptoms caused by chilling (Sun 1971; Van Lelyveld and De Bruyn 1977). Ascorbic acid may be oxidised before the phenols are oxidised by polyphenol oxidase which would then cause browning to occur. The highest levels of ascorbic acid are found in the periphery and the top of the fruit. This gradient was thought to explain in part the occurrence of black heart symptoms initially around the core (Hammer and Nightingale 1946; Miller and Hall 1953).

Ascorbic acid was corelated to pH and TA only in shrinkwrapped fruits. Hammer and Nightingale (1946) and Miller and Hall (1953) found an identical gradient of ascorbic acid to titratable acidity within the pineapples. Ascorbic acid was correlated to the soluble solids content (SSC) only in the waxed and shrinkwrapped samples. Ascorbic acid also negatively correlated to the fruit texture for most treatments.

L and (a+b) values were also correlated to pH only in waxed and control samples stored at 15°C, 20°C and ambient temperature but not for samples stored at 10°C. Van Lelyveld and De Bruyn (1976) found IB-affected fruits had lower citric and malic acids; this was thought to be due to assimilation in the biogenesis of ascorbic acid through citric acid cycle and glucuronate and gulonate pathways. Colour was also found to be correlated to TSS, especially in the control fruits. Various researchers (Van Lelyveld and De Bruyn 1976; Abdullah and Rohaya 1983) found IB to lower the TSS values (mainly sucrose and fructose) in pineapples but found no correlationship of IB with pH or TA.

Weight loss was found to be negatively correlated to pulp texture and ascorbic acid con-

tents for the pineapples under most conditions (Table 3) and may relate to the gas/moisture permeability of the surface coating or packaging used.

CONCLUSION

The weight loss of pineapples was effectively controlled by LDPE shrinkwrapping, and to a lesser extent by paraffin waxing. Fruit firmness was effectively retained by shrinkwrapping. Paraffin waxing at the concentration used caused lesions on the skin and was thus detrimental to the fruit quality. Paraffin was most effective in preventing IB and retaining the colour of pineapple pulp. The ascorbic acid content of paraffin-waxed fruits was generally higher than that in untreated fruits except for pineapples stored at room temperature. This is thought to be due to the reduced destruction of ascorbic acid with reduced oxygen content in the tissues of the paraffin-treated pineapples. Paraffin waxing and shrinkwrapping also reduced the acidity of the pineapples substantially in comparison to the untreated fruits.

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Inbreeding Depression and Heterosis in Sweet Corn Varieties Manis Madu and Bakti-1

GHIZAN BIN SALEH*, MOHD. RAFII YUSOP** and YAP THOO CHAI*

**Department of Agronomy and Horticulture, Faculty of Agriculture, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia*

***Palm Oil Research Institute of Malaysia (PORIM) Research Station, 86000 Kluang, Johor Darul Takzim, Malaysia*

ABSTRAK

Famili-famili progeni S_1 dan penuh-sib yang dibentuk dari penyendirian dan kacukan antara varieti jagung manis Manis Madu dan Bakti-1 telah dinilai untuk menganggarkan kemelesetan penginbredan dan heterosis dalam populasi. Penyendirian telah menyebabkan berlakunya pengurangan yang bererti di dalam nilai ukuran untuk semua ciri yang diukur dalam kedua-dua populasi penyendirian, kecuali untuk ciri masa pentaselan yang menunjukkan pertambahan pada nilainya. Anggaran heterosis berdasarkan nilai pertengahan induk untuk ciri-ciri yang dinilai ialah di antara -2.83% dan 22.34% bagi populasi progeni kacukan Manis Madu X Bakti-1 (MMB1), dan di antara -2.65% dan 16.57% bagi populasi progeni kacukan Bakti-1 X Manis Madu (B1MM). Kedua-dua varieti menunjukkan potensi baik untuk digunakan sebagai induk dalam kacukan antara populasi-populasi maju atau warisan-warisan inbred yang terbentuk darinya.

ABSTRACT

S_1 and full-sib progeny families developed from selfing and crossing between Manis Madu and Bakti-1 sweet corn varieties were evaluated to estimate inbreeding depression and heterosis in the populations. Selfing has caused a significant decrease in the measurements of all characters taken in both selfed populations, except for days to tasseling which has shown an increase. Midparent heterosis estimates for the characters evaluated ranged from -2.83% to 22.34% for the Manis Madu X Bakti-1 cross progeny population (MMB1), and from -2.65% to 16.57% in the Bakti-1 X Manis Madu cross progeny population (B1MM). The two varieties revealed good potential to be used as parents for crosses between improved populations or inbred lines developed from them.

Keywords: *Zea mays*, sweet corn, inbreeding depression, heterosis

INTRODUCTION

Inbreeding is the process of mating between genetically related individuals. Selfing is the strongest form of inbreeding. As a consequence of selfing, recessive genes, earlier masked in the heterozygous forms, become homozygous. These genes, if conferring to undesirable phenotypes, will result in the deterioration of the succeeding generations. In cross-pollinated crops which do not have self-incompatibility problems, like corn, however, inbred lines for hybrid varieties are developed through selfing.

Extensive studies on inbreeding depression in corn have indicated that selfing is important in inbred development because it leads to rapid gene homozygosity, and desirable dominant genes can be accumulated while the undesirable ones are eliminated. The performance of inbred lines or lines produced from selfing decreased drastically, resulting in yield reduction, increase in the number of stunted plants, reduced plant resistance to pests and diseases and reduced growth rate (Genter 1971; Harris *et al.* 1972; Hallauer and Sears 1973; Cornelius and Dudley 1974; Good and Hallauer 1977; Saleh *et al.* 1990).

Heterosis or hybrid vigour is the effect which is opposite to inbreeding depression. From the aspect of quantitative genetics, heterosis is the value or measurement of a hybrid beyond the average value of the two parents. However, in plant breeding a hybrid that performs better than the better parent is desired. Heterosis estimates for corn yield based on the mid-parental values in crosses between populations have been reported, including 19.2% from the variety cross of 'Jarvis' X 'Indian Chief' (Moll and Stuber 1971), 14.4% from the cross of 'Iowa Stiff Stalk Synthetic' X 'Iowa Corn Borer Synthetic No. 1'

(Eberhart *et al.* 1973), 6.0% from the cross of 'Teko Yellow' X 'Natal Yellow Horse Tooth' (Gevers 1975), 39.3% from the cross of 'KII' X 'EC573' (Darrah *et al.* 1978) and 14.9% from the cross of 'BSSS' X 'BSCB1' (Martin and Hallauer 1980).

The objectives of this study were to determine inbreeding depression in the S_1 families, and to estimate heterosis revealed by the full-sib progenies developed from the crosses, in the first cycle of a recurrent selection programme involving the sweet corn varieties Manis Madu and Bakti-1.

MATERIALS AND METHODS

The study was conducted at the Faculty of Agriculture Research Plot, Universiti Pertanian Malaysia, Serdang, Selangor. The open-pollinated local sweet-corn varieties, Manis Madu and Bakti-1 were used as source populations.

This study was part of the simple and reciprocal recurrent selection programmes undertaken on the two varieties. In the first planting season, self-pollinations and full-sib crosses were carried out to develop selfed and full-sib progeny families. The selfed-progeny families were MMS_1 for Manis Madu and $B1S_1$ for Bakti-1, while the full-sib families were MMB1 from the Manis Madu X Bakti-1 cross, and B1MM from its reciprocal cross. For this purpose, the source populations, Manis Madu and Bakti-1 were planted at the density of 100 cm x 50 cm, and female inflorescences of 250 plants of each population were hand-pollinated to form each of the progeny families. The total number of uncontaminated families formed were 195 MMS_1 families, 197 $B1S_1$ families, 176 MMB1 families and 189 B1MM families.

In the second planting season, evaluations of the S_1 and full-sib families were conducted in a randomised complete block design, with two replications. The planting density used was 75cm x 25 cm. The original source populations were used for comparison, where one row of the respective source population was planted for every six progeny rows in the S_1 family evaluation, while one row of each of the source populations was planted for every six progeny rows in the full-sib progeny evaluation. Every progeny-row in a replication comprised twelve plants. Six plants from each family were taken at random as samples for the analysis of data. Seven plant characters and yield components were studied.

Estimates of inbreeding depression in the selfed populations were determined as follows:

$$\text{Inbreeding depression} = \frac{\bar{S}_1 - \bar{S}_0}{\bar{S}_0};$$

where $\bar{S}_0$ = S_0 population mean, and
$\bar{S}_1$ = S_1 population mean.

Estimates of heterosis in the cross-progeny populations were determined as follows:

$$\text{Mid-parent heterosis} = \frac{\bar{F}_1 - \overline{MP}}{\overline{MP}};$$

$$\text{Better-parent heterosis} = \frac{\bar{F}_1 - \overline{HP}}{\overline{HP}};$$

where $\bar{F}_1$ = performance of full-sib crossed-progeny,
$\overline{MP}$ = mean of the performance of the two parental populations, and
$\overline{HP}$ = performance of the better parental population.

RESULTS AND DISCUSSION

Inbreeding Depression

The estimates of inbreeding depression for the selfed populations are shown in Table 1. In both selfed populations, the estimate of inbreeding depression was highest for fresh largest-ear weight per plant, followed by ear length, ear height, plant height, ear diameter and days to tasselling. Contrary to the other characters, inbreeding depression for days to tasselling was shown by the increase in the magnitude of the measurement.

There were substantial differences in the inbreeding depression estimates between MMS_1 and $B1S_1$ populations. For all characters evaluated, except days to tasselling, estimates of inbreeding depression in MMS_1 were higher than those in $B1S_1$. The estimates in population MMS_1 and $B1S_1$ respectively, were -33.58% and -21.17% for fresh largest-ear weight per plant, -20.48% and -11.10% for ear length, -16.75% and -10.18% for ear height, -14.77% and -8.43% for plant height, and -8.73% and -6.57% for ear diameter. Selfing caused lateness in tasselling in both populations. There was no obvious difference in inbreeding depression for days to tasselling between the two selfed populations, i.e. +3.35% in MMS_1 and +3.24% in $B1S_1$.

One generation of selfing in the two populations reduced plant and ear size and yield. This was due to the unmasking of the recessive alleles of the genes responsible for the control of these characters as the result of selfing. The accumulation of recessive alleles in the homozygous form due to selfing, thus, resulted in plant and ear size, and yield reductions. Similar results were reported by Cornelius and Dudley (1974)

TABLE 1
Estimates of inbreeding depression in MMS_1 and $B1S_1$ populations

Character	Population	Mean ± SE		Inbreeding depression (%)
		S_0	S_1	
Fresh largest-ear weight per plant (g)	MM	198.84 ± 3.10	132.07 ± 2.79	- 33.58**
	B1	226.48 ± 2.91	175.90 ± 2.72	- 22.23**
Fresh dehusked largest-ear weight per plant (g)	MM	133.72 ± 2.15	84.95 ± 1.96	- 36.47**
	B1	na	na	na
Days to tasselling (days)	MM	53.15 ± 0.12	54.93 ± 0.13	+ 3.35**
	B1	49.10 ± 0.10	50.69 ± 0.13	+ 3.24**
Ear diameter (mm)	MM	40.54 ± 0.27	37.00 ± 0.21	- 8.73**
	B1	40.56 ± 0.30	37.85 ± 0.20	- 6.68**
Ear length (cm)	MM	13.87 ± 0.18	11.03 ± 0.16	- 20.48**
	B1	14.19 ± 0.20	12.58 ± 0.17	- 11.35**
Plant height (cm)	MM	146.15 ± 1.16	124.57 ± 1.14	- 14.77**
	B1	183.41 ± 1.20	167.94 ± 1.14	- 8.43**
Ear height (cm)	MM	70.50 ± 0.85	58.61 ± 0.83	- 16.75**
	B1	90.86 ± 0.96	81.61 ± 0.86	- 10.18**

na Data not available
** Significantly different from zero at $p < 0.01$

and Good and Hallauer (1977), who found reduction in yield and other characters, except for tasselling date. Lateness in tasselling as a consequence of selfing showed that the alleles responsible for earliness are dominant to those responsible for lateness. Lateness in tasselling due to inbreeding was also reported by Marsum (1972), Sears and Hallauer (1973), Cornelius and Dudley (1974).

The highest estimates of inbreeding depression were obtained from yield characters, i.e. fresh largest-ear weight per plant and fresh dehusked largest-ear weight per plant. Similar results were reported by Genter (1970), who found that yield characters experienced a higher rate of inbreeding because they were controlled by a higher number of genes. High rates of inbreeding depression were also reported by Genter (1971), Harris *et al.* (1972), Marsum (1972), Hallauer and Sears (1973), Cornelius and Dudley (1974).

The difference in the estimates obtained between populations MMS_1 and $B1S_1$ could be due to the difference in the nature and number of genes involved in the control of the characters in the two source populations. It could also be due to the interactions between the genotype and the environment, because the two populations were evaluated separately at different times. Genter (1971) also reported that different corn populations gave different inbreeding depression estimates. Harris *et al.* (1972) also indicated that differences in inbreeding depression estimates were caused by genetic and environmental factors.

Heterosis

Estimates of heterosis obtained from mean performance of all families in each of the populations, MMB1 and B1MM are shown in Table 2. In general, the MMB1 population showed higher heterosis compared to the B1MM population.

Based on mid-parental value in the MMB1 population, fresh largest-ear weight per plant showed the highest heterosis estimate (22.34%), followed by fresh dehusked largest-ear weight per plant (19.13%), ear height (18.97%), plant height (13.11%), ear length (9.63%) and ear diameter (3.95%). In the B1MM population however, the highest estimate of heterosis, based on the mid-parental value was shown by fresh dehusked largest-ear weight per plant (16.57%),

TABLE 2
Estimates of heterosis in MMB1 and B1MM populations

Character	Cross Pop.	Mean ± SE			Heterosis (%)	
		B1	MM	F_1	Mid-Parent	Better-Parent
Fresh largest-ear weight per plant (g)	MMB1	152.25 ± 3.01	138.95 ± 2.80	178.12 ± 3.05	22.34 **	16.99 **
	B1MM	175.60 ± 3.25	198.79 ± 3.82	195.40 ± 3.61	4.38 *	- 1.71
Fresh dehusked largest-ear weight per plant (g)	MMB1	109.43 ± 2.06	97.48 ± 1.98	123.25 ± 2.19	19.13 **	12.63 **
	B1MM	105.22 ± 2.03	115.68 ± 2.13	128.75 ± 2.43	16.57 **	11.30 **
Days to tasselling (days)	MMB1	54.16 ± 0.14	54.27 ± 0.15	52.68 ± 0.13	- 2.83 *	- 2.73 **
	B1MM	56.18 ± 0.19	55.40 ± 0.17	54.31 ± 0.17	- 2.65 *	- 1.97
Ear diameter (mm)	MMB1	36.29 ± 0.17	36.05 ± 0.15	37.60 ± 0.20	3.95 *	3.61 *
	B1MM	38.92 ± 0.19	41.01 ± 0.22	40.07 ± 0.21	0.26	- 2.29 *
Ear length (cm)	MMB1	11.10 ± 0.11	10.92 ± 0.10	12.07 ± 0.13	9.63 **	8.74 **
	B1MM	12.05 ± 0.13	12.60 ± 0.14	12.72 ± 0.15	3.20 *	0.95
Plant height (cm)	MMB1	135.23 ± 1.35	126.47 ± 1.20	148.01 ± 1.29	13.11 **	9.45 **
	B1MM	145.96 ± 1.40	141.83 ± 1.36	147.88 ± 1.47	2.77 *	1.32
Ear height (cm)	MMB1	56.73 ± 0.73	56.04 ± 0.76	67.08 ± 0.89	18.97 **	18.24 **
	B1MM	60.96 ± 0.80	63.13 ± 0.86	67.96 ± 1.00	9.53 **	7.65 **

**,* Significantly different from zero at $p < 0.01$ and 0.05, respectively.

followed by ear height (9.57%), fresh largest-ear weight per plant (4.38%), ear length (3.20%), plant height (2.77%) and ear diameter (0.26%). Heterosis for days to tasselling did not show a large difference between populations (-2.83% for MMB1, and -2.65% for B1MM).

Based on better-parent value in the population MMB1, highest heterosis estimate was shown by ear height (18.24%), followed by fresh largest-ear weight per plant (16.99%), fresh dehusked largest-ear weight per plant (12.63%), plant height (9.45%), ear length (8.75%) and ear diameter (3.61%). In the B1MM population, the highest estimate of heterosis was obtained for fresh dehusked largest-ear weight per plant (11.30%), followed by ear height (7.65%), plant height (1.32%) and ear length (0.95%). For fresh largest-ear weight per plant and ear diameter, heterosis estimates were negative (-1.71% and -2.29%, respectively), indicating that the crossed population did not perform better than the better parent for these characters. Better-parent heterosis estimates for days to tasselling were -2.73% for MMB1 and -1.97% for B1MM.

In both populations, many families showed very high heterosis estimates. In population MMB1, mid-parent heterosis and better-parent heterosis in individual families were as high as 99.18% and 90.48% respectively, for fresh largest-ear weight per plant. Similarly, in population B1MM, the highest values shown by a family for this character were 68.86% for mid-parent heterosis, and 59.01% for the better-parent heterosis. For fresh dehusked largest-ear weight per plant, the estimates for the individual families were as high as 86.84 and 76.64% for mid-parent heterosis and better-parent heterosis, respectively in population MMB1; the two estimates were 93.53 and 84.78%, respectively for the population B1MM. Heterosis estimates for ear diameter and ear length for families were as high as 21.37% (mid-parent) and 24.23% (better-parent), in MMB1, and as high as 24.23% (mid-parent) and 21.07% (better-parent), in B1MM. A similar trend was found for the other characters measured.

Positive estimates of heterosis obtained for yield components including fresh dehusked largest-ear weight per plant and ear length, as shown in this study were also reported by Peterniani and Lonnquist (1963), Moll and

Stuber (1971), Eberhart *et al.* (1973), Gevers (1975), Darrah *et al.* (1978), Martin and Hallauer (1980). The differences in the estimates of heterosis were due to the different populations used.

Both progeny populations produced tassels earlier than their respective parents, as evidence of heterosis. This showed that the alleles which determine earliness in tasselling are dominant to those responsible for lateness, as reported by Robinson *et al.* (1949). Positive heterosis for plant and ear heights were also reported by Pasev and Trifunovic (1968).

The presence of some degree of reciprocal effects was also detected, where Manis Madu variety gave a higher heterotic effect in the progenies, if used as the female parent (MMB1), compared to when it was used as the male (B1MM).

The presence of substantial heterosis in the progenies of crosses between Manis Madu and Bakti-1 indicates that the two varieties have good potential as parents for population crosses or inbred development for hybrid variety production. A very high heterosis revealed by many families indicates that recombination of the promising lines selected from these populations in the later phase of the recurrent selection programme, should help develop new populations with higher combining ability with the reciprocal populations.

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Profile of Fatty Acid Contents in Malaysian Freshwater Fish

K. ENDINKEAU and TAN KIM KIEW
Department of Chemistry
Faculty of Science and Environmental Studies
Universiti Pertanian Malaysia
43400 UPM Serdang, Selangor Darul Ehsan
Malaysia.

ABSTRAK

Sembilan spesies ikan air tawar Malaysia telah dianalisis kandungan lipid dan asid lemaknya. Empat spesies ikan yang biasa dimakan oleh penduduk tempatan didapati mengandungi lemak sangat tinggi (julatnya 11-17% berat basah). Dalam semua ikan air tawar yang dikaji kandungan asid lemak tak tepunya melebihi asid lemak tepu. Nisbah asid lemak tak tepu/tepu adalah di antara 1.2 hingga 2.3. Asid kumpulan omega-3, pada amnya rendah dalam semua spesies dikaji, kecuali belut sawah yang mengandungi asid C22:6w3 sangat tinggi (9.4 g/100g minyak). Jumlah ini setanding dengan nilai-nilai yang terdapat pada ikan salmon, cod dan herring. Oleh itu penternakan belut sawah dan pengekstrakan minyaknya berpotensi dieksploit secara komersial. Pecahan fosfolipid bagi semua spesies yang dianalisis, kecuali jelawat mempunyai nisbah politak tepu/tepu lebih daripada 1.0.

ABSTRACT

Nine species of Malaysian freshwater fish were analysed for their lipid and fatty acid contents. The results show that 4 species of fish commonly consumed by local people contained significantly high levels of fat (range 11-17% of wet weight). Malaysian freshwater fish analysed also contained high levels of unsaturated acids compared to saturated acids. The ratio of unsaturates/saturates ranged from 1.2 to 2.3. The omega-3 acids were generally low in most species analysed except for the belut sawah which contained significantly high levels in C22:6w3 (9.4/100g oil). This quantity is comparable to that of salmon, cod and herring and thus warrants consideration for commercial exploitation. Phospholipid fractions of all fish analysed (except jelawat) had polyunsaturates/saturates ratios greater than 1.0.

Keywords: freshwater fish, fatty acid, phospholipid, fish lipid

INTRODUCTION

Fish is a major source of protein in the Malaysian diet. The freshwater fish industry in this country is emerging rapidly due to technology advancement and government support. However, for some reason, marine fish is preferred to freshwater fish among Malaysians. Demand for freshwater fish has remained low in the past few years. Lack of information on the nutritional value of freshwater fish could be one of the main reasons for the above situation.

The importance of fish in maintaining health was realised after studies were conducted on Greenland Eskimos and several Japanese populations (Dyerberg *et al.* 1975; Dyerberg and Bang 1979; Dyerberg 1982; Kagawa *et al.* 1982). The studies showed very little incidence of coronary heart disease among the subjects studied. These observations have been correlated with high intake of fish and marine organisms in their diet. The marine products consumed by these people are rich in polyunsaturated fatty acids (PUFA), particularly in eicosapentaenoic (EPA, C22:5ω3) and docosahexaenoic (DHA, C22:6ω3) acids. The effects of these acids on chronic diseases are well documented (Dyerberg *et al.* 1978; Jones and Davies, 1982; Kenneth 1986; Simopoulos *et al.* 1986; Kinsella 1988). Recent research has shown that polyunsaturated fish oils could lower serum triglyceride and cholesterol levels, and also help to prevent blood clotting (Dyerberg 1986; Herold and Kinsella 1986).

Most studies in the past have been carried out on northern hemisphere cold water fish (Ackman 1982) such as mackerel, herring, sardine and cod. These species are known to be excellent sources of PUFA, especially the omega-3 fatty acids. Recently, fish obtained from tropical waters were also found to be rich in polyunsaturated fatty acids (O'Dea and Sinclair 1982).

A similar result has been reported by Gibson (1983) for the fish caught in temperate waters of southern Australia. Analysis of Malaysian freshwater fish, however, has never been published before, although there is a report on marine fish (Gibson *et al.* 1984). The present study was conducted to quantify PUFA of some commonly reared freshwater fish such as tilapia, lampam jawa, siakap, etc. in Malaysia to determine the nutritional value of these fishes.

MATERIALS AND METHODS

All fish were bought fresh from the local market at separate times during the course of this study. Fish species were identified by an officer from the State Department of Fisheries. Prior to analysis, fish fillets were obtained by carefully cutting the fish lengthwise along the backbone to obtain maximum flesh without traces of backbone. Two to three fish were used each time, and fillets were mixed and cut into small portions before analysis.

Samples of fish fillets (50 g) were homogenized in a blender for 2 min with a mixture of chloroform-methanol (150 ml, 1:2 v/v), according to the method of Bligh and Dyer (1959). Butylated hydroxytoluene (BHT) at a concentration of 0.2% (of the fillet) was added at the beginning of extraction to prevent oxidation. The extract was filtered and evaporated to dryness *in vacuo*, on a rotary evaporator at 40°C. The resulting lipid fraction was weighed and stored at -18°C for further analysis.

Lipid samples were converted to constituent fatty acid methyl esters (FAME) by refluxing the lipid (50 mg) in 5 ml of reagent consisting of concentrated sulphuric acid-toluene-methanol (1:10:20 v/v) for one hour at 90°C, according to the method of Hammond (1987). After cooling, water (3 ml), hexane (3 ml) and internal standards (6µl) (C15 and C19 of Sigma Chem. Co.) were added. The hexane layer was recovered, dried over anhydrous Na_2SO_4 and the FAME were ready for injection.

Routine analyses of FAME were performed by gas chromatography. The esters were analysed using a gas chromatograph (Shimadzu GC-9A provided with an FID and coupled with a Shimadzu C-R3A computerised integrator). A fused silica capillary column (30 m x 0.53 mm id) of Supelcowax-10 with 0.50 µm film thickness (Supelco, Inc.) was used. The oven temperature was programmed from 100°C to 240°C at a ramp rate of 5°C/min, after an initial isothermal period of 2 min and was held for 10 min after final temperature. The detector and injector port temperatures were 280°C and 250°C respectively. The carrier gas was helium, set at a flow rate of 50 ml/min. Identification of FAME was based on comparison of retention times between unknown peaks and those of authentic standards (Sigma Chem. Co). Individual esters were quantified by the internal standard method. Duplicate injections were carried out on each FAME sample.

Phospholipid fraction of extracted fish lipid was separated by column chromatography on silica gel (70-230 mesh). The lipid (3 g) was dissolved in hexane and added to the column, using chloroform, acetone and methanol as eluents respectively (Carroll 1976). The phospholipid obtained was concentrated with a rotary evaporator, weighed and esterified as before.

All organic solvents used in this study were reagent grade and used without further purification.

RESULTS AND DISCUSSION

Nine species of commercially reared Malaysian freshwater fish were analysed. The fish are usually reared in captivity and fed with commercial feed. The species chosen are either commonly eaten by Malaysians or are very popular among seafood lovers. All fish samples used were mature and at normal harvesting size.

The lipid contents of all selected Malaysian freshwater fish are shown in Table 1 and ranged from 1.8 to 17.8 g/100g wet weight. Four species known locally as keli *(Clarius* sp.*)*, lampam jawa *(Puntius gonionotus)*, lee koh *(Cyprinus carpio innaeus)* and tilapia *(Oreochromis* sp.*)* contained relatively high levels of lipid, 13.0%, 14.8%, 17.8% and 11.0% of wet fillet respectively. In contrast, siakap *(Lates calcarifer)* had a much lower fat content (2.0%). Haruan *(Channa striatus)* which is believed to have healing properties (Mohsin and Ambak 1983) and in high demand was also low in fat (2.0%). The most expensive local fish which is noted for its delicate flavour, jelawat *(Leptobarbus hoevenii)*, was found to contain 7.9% fat. The lipid content obtained from this study is generally high compared to those reported for Malaysian marine fish (Gibson *et al.* 1984) and Australian tropical water fish (O'Dea and Sinclair 1982). The previous highest values reported for Malaysian and Australian fish were 3.9 and 7.8 g/100 g wet weight respectively. The high fat levels observed in this study suggest that the freshwater fish are fed the right diet whereas marine fish rely mainly on limited food available from the surroundings. The observed variables in

TABLE 1
Local, common and scientific names and lipid content of fish (g/100g fillet)

Local Name	Common Name	Scientific Name	Lipid Content*
Belut sawah	Rice-field eel	*Monopterus alba* (Zuiew)	1.77
Jelawat	Sultan fish	*Leptobarbus hoevenii* (Bleeker)	7.90
Kap rumput	Grass carp	*Ctenopharyngodan idellus* (C&V)	7.52
Keli	Walking fish	*Clarius* spp.	12.96
Lampam jawa	Javanese carp	*Pluntius gonionotus* (Bleeker)	14.82
Lee koh	Common carp	*Cyprinus carpio* Linnaeus	17.76
Siakap	Sea bass, Sea perch	*Lates calcarifer* (Bloch)	1.97
Tilapia	African bream	*Oreochromis* spp.	11.01
Haruan	Snake head	*Channa striatus* (Bloch)	2.10

* mean of two determinations

lipid content among the species studied (ranging from 1.8 - 17.8%) could be attributed to differences in type of diets, age of fish, the habitat, the activity pattern and the species (Kinsella 1987).

Normal separation of fatty acid methyl esters (FAME) obtained from fish lipid samples is illustrated in *Fig. 1*. The chromatograms show some major peaks derived from FAME of kap rumput and its phospholipid fraction. The peaks are well separated on Supelcowax-10 and thus give a better area integration. The percentage of the major fatty acids composition in fish lipids is given in Table 2. Palmitic acid (C16:0) was the major component of the saturated fatty acids followed by stearic acid (C18:0) in all species analysed. Keli (*Clarius* sp.) displayed the highest palmitic acid content (15.2 g/100 g fish oil). The other saturated fatty acids, including C12:0 and C14:0, were only minor components. In most species examined, oleic acid (the only monounsaturated acid quantified) was found to be a major constituent of freshwater fish (more than 17%). In contrast, haruan, siakap and belut sawah only contained 4.8, 7.3 and 4.8% oleic acid respectively.

Linoleic acid (C18:2ω6), was the predominant fatty acid in the polyunsaturated components or ω6 group in most of the species studied. Its concentration ranged from 0.6 to 11.5 g/100 g fish oil. Lampam jawa was the highest in C18:2 (11.5 g) followed by tilapia (11.4 g) and lee koh (10.0 g), whilst belut sawah was the lowest (0.6 g). Another ω6 acid, arachidonic acid (C20:4ω6) was also present at significant levels in the fishes studied. Siakap and haruan were among the highest in arachidonic acid content, which were 2.0 g (ca:6.1% of total fatty acids) and 2.3 g/100 g oil (ca:11.0%) respectively. The value obtained from haruan was comparable to that of Gibson *et al.* (1984) report on Malaysian shrimp (11.8%) (the highest arachidonic content in Malaysian marine fish analysed). The values obtained both from this study and by Gibson were higher than those from cold-water fish of the northern hemisphere (Ackman 1982). In this aspect, haruan may be considered as a potential source of arachidonic acid. This acid plays a major role in fat metabolism in the human body. It is the most important precursor of eicosanoids which have a physiological effect on the vascular system (Kinsella 1986).

An interesting result was observed in belut sawah. Although the fat content was low (1.8%), belut sawah contained a high concentration of docosahexaenoic acid (DHA) (C22:6ω3). The value obtained in this study (9.4 g/100 g oil)or ca. 0.17 g/100 g fillet was comparable to that of sockeye salmon (0.71 g/100 g tissue), Pacific herring (0.75 g/100 g) and cod (0.19 g/100 g) (Kinsella 1987). These species have won wide recognition as good sources of ω3 acids. A further analysis of belut of different sizes was carried out. The results show that the level of DHA increased according to size of fish. A similar result was also obtained for fat contents (Table 3). This result may lead to the exploitation of ω3 acid in belut sawah commercially. *Fig. 2* shows a significant peak of DHA derived from FAME of

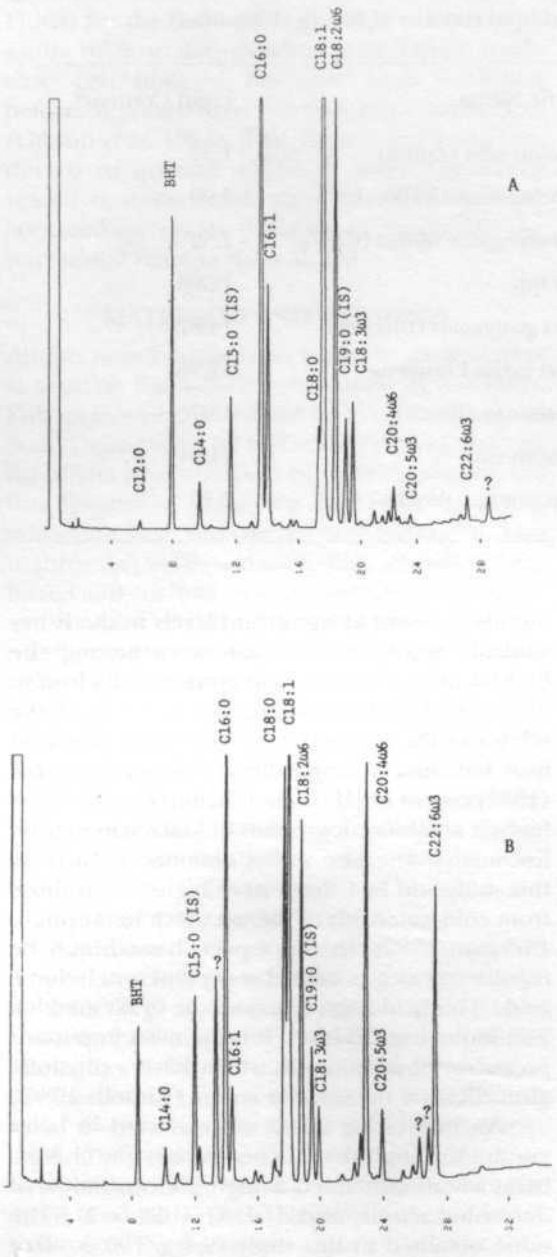

Fig. 1: Separation of fatty acid methyl esters derived from (A): total lipid, and (B): phospholipid fraction of kap rumput on a Supelcowax-10 capillary column (30 m x 0.53 mm id, Atten. = 1). Note the peak heights of C20:4ω6 and C22:6ω3 in phospholipid fraction.

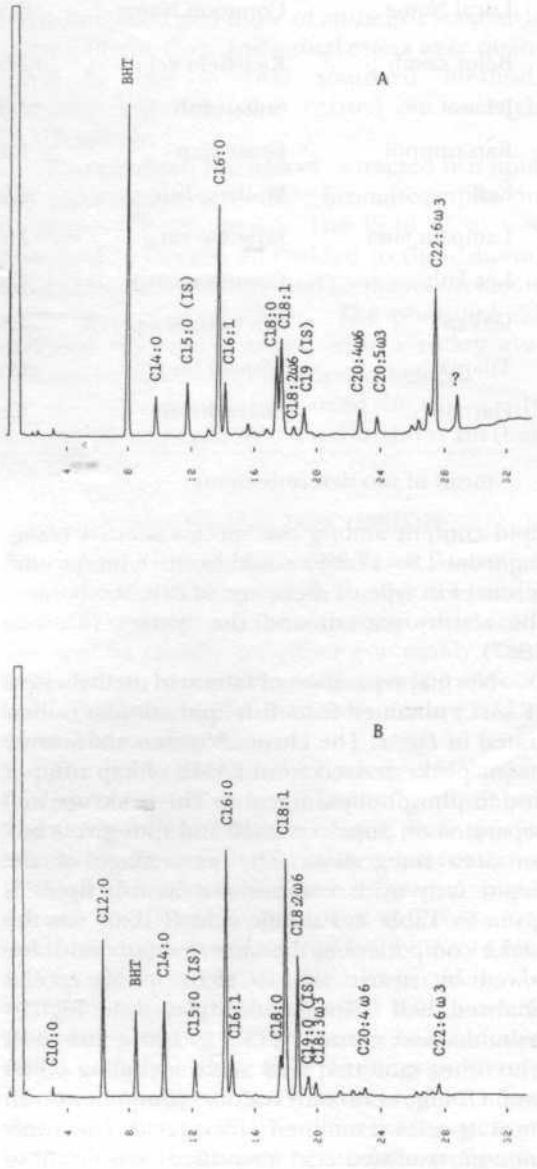

Fig. 2: Separation of fatty acid methyl esters obtained from lipids of (A): belut sawah, amd (B): tilapia on a Supelcowax-10 (Atten. =3). Note the peak heights of C22:6ω3 in (A) and (B), and the appearance of more saturated acid peaks at the lower molecular weight region (B).

belut (A) compared to tilapia (B). Additionally, siakap (6.1 g) and jelawat (3.0 g) were also found to contain considerable amounts of DHA. In all cases, the content of eicosapentaenoic acid (EPA) (C20:5ω3) was much lower compared to DHA.

Generally, the fatty acids of the fish examined in this study showed a higher unsaturated, rather than saturated acid content. The ratio of unsaturates/saturates ranged from 1.2 to 2.3 (Table 2). Three species, namely kap rumput (2.3), lampam jawa (2.1) and lee koh (2.3) had a

TABLE 2
The average fatty acid composition of total lipids (g/100 g fish oil)

Fatty acid	Belut sawah	Jelawat	Kap rumput	Keli	Lampam jawa	Lee koh	Siakap	Tilapia*	Haruan
Saturates									
12:0	nd	0.08	0.10	nd	0.06	0.17	0.09	4.61	nd
14:0	0.98	1.20	0.65	0.42	0.52	0.68	1.23	3.89	0.34
16:0	6.76	11.82	11.47	15.19	12.39	13.74	9.14	11.70	6.55
18:0	3.35	4.69	1.39	2.41	2.54	1.96	4.17	2.40	2.24
20:0	nd	nd	nd	nd	nd	nd	0.08	0.09	nd
Monounsaturates									
18:1	4.75	17.19	18.56	18.15	19.98	26.94	7.20	15.06	4.73
Polyunsaturates									
18:2 ω6	0.55	6.76	9.22	9.65	11.54	9.96	1.00	11.44	2.05
18:3 ω3	0.14	1.30	2.25	0.24	0.65	0.31	nd	0.93	0.42
18:4 ω3	0.12	0.22	0.08	0.07	0.10	0.16	0.08	0.10	0.04
18:4 ω6	1.23	1.38	0.58	0.36	0.44	0.68	2.00	0.46	2.28
18:5 ω3	0.84	0.85	0.19	0.06	nd	0.20	1.56	0.11	0.09
18:6 ω3	9.37	3.03	0.55	0.49	0.31	0.34	6.13	0.77	2.11
Σ saturates	11.09	17.79	13.61	18.02	15.51	16.55	14.71	22.99	9.13
Σ unsaturates	17.00	30.73	31.34	29.02	33.02	38.59	17.97	28.87	11.72
ω3 acids (C20: 5 + C22:6)	10.21	3.88	0.74	0.55	0.31	0.54	7.69	0.88	2.20
ratio $\frac{\Sigma \text{ unsaturates}}{\Sigma \text{ saturates}}$	1.53	1.73	2.31	1.61	2.13	2.33	1.22	1.26	1.28

*Tilapia consists C10:0 = 0.30 g/100g fish oil nd = not detectable

TABLE 3
Comparative fatty acid (g/100 g fish oil) composition of belut in different sizes

Fatty Acid	Sizes of Belut		
	420 g	280 g	100 g
Saturates			
14:0	0.98	0.16	nd
16:0	6.76	3.71	1.67
18:0	3.35	1.94	1.34
20:0	nd	nd	nd
Monounsaturates			
18:1	4.75	2.04	0.89
Polyunsaturates			
18:2 ω6	0.55	0.37	0.43
18:3 ω3	0.14	nd	nd
18:4 ω3	0.12	nd	nd
20:4 ω6	1.23	3.06	1.92
20:5 ω3	0.84	0.39	0.21
20:6 ω3	9.37	4.10	1.92
Total lipid* (g/100g fillet)	1.77	1.14	0.90

*mean of two determinations

ratio of above 2; thus the fats of these fish can be classified as unsaturated. *Fig. 1(A)* shows a typical FAME chromatogram of kap rumput where more peaks appeared in the region of higher molecular weight (unsaturates). On the other hand, the FAME of tilapia *(Fig.2B)* contained more peaks on the lower molecular weight region (saturates).

The composition of fatty acids in the phospholipid fraction of five species analysed showed that this fraction was high in PUFA (Table 4). In some cases (siakap, tilapia and kap rumput) the total percentage of arachidonic acid and DHA was almost equal to those of saturated components, i.e. palmitic and stearic acids. Again, phospholipid of belut sawah was the highest in DHA (40.1%), followed by siakap (30.7% of total fatty acid). *Fig. 1(B)* shows a FAME chromatogram of phospholipid of kap rumput. Peaks correspond to major PUFA, especially the DHA and EPA which are relatively higher compared to those of saturated acids. When compared to total lipid fractions the phospholipid fractions contain higher percentages of PUFA (not including monounsaturated acid, C18:1), the percentages being in the range of 48.9 - 59.4%, while the corresponding levels in total lipid range from 36.7 - 45.0% of total fatty acids (Table 5). All ratios of polyunsaturated/saturated were above 1.3 except jelawat (0.7). In contrast, the total lipid fractions have a ratio less than 1 (Table 5). This

TABLE 4
Fatty acid composition of phospholipids in percentage of total fatty acids attained*

Fatty acid	Belut sawah	Jelawat	Kap rumput	Siakap	Tilapia#
Saturates					
12:0	0.12(0.001)	0.15(0.001)	nd(nd)	nd(nd)	0.98(0.005)
14:0	0.86(0.007)	3.13(0.021)	0.81(0.005)	1.16(0.005)	1.96(0.010)
16:0	11.59(0.094)	28.32(0.190)	15.19(0.094)	20.00(0.086)	15.32(0.078)
18:0	27.99(0.227)	9.54(0.064)	13.49(0.058)	13.49(0.058)	20.43(0.104)
20:0	nd(nd)	0.15(0.001)	nd(nd)	nd(nd)	0.39(0.002)
Monounsaturates					
18:1	nd(nd)	29.06(0.195)	19.39(0.195)	16.74(0.072)	11.79(0.060)
Polyunsaturates					
18:2 ω6	1.23(0.010)	14.61(0.098)	16.32(0.101)	3.49(0.015)	12.97(0.066)
18:3 ω3	nd(nd)	3.58(0.024)	2.75(0.017)	nd(nd)	0.59(0.003)
18:4 ω3	nd(nd)	0.60(0.004)	nd(nd)	0.23(0.001)	nd(nd)
20:4 ω6	15.17(0.123)	2.83(0.004)	15.51(0.096)	9.07(0.039)	20.04(0.102)
20:5 ω3	2.96(0.024)	2.68(0.018)	2.75(0.017)	5.12(0.022)	0.59(0.003)
22:6 ω3	40.07(0.325)	5.37(0.035)	13.73(0.085)	30.70(0.132)	14.73(0.075)

*Values in brackets are g/100 g fish oil
# Phospholipid of tilapia consists C10:0 = 0.20% (0.001g/100 g fish oil)
nd = not detectable

TABLE 5
Comparative analysis of fatty acid composition in total lipid and phospholipid
(results are expressed as percentage of total fatty acids attained)

	Total Lipid			Phospholipid		
Fish	Polyunsaturates*	Saturates	ratio#	Polyunsaturates*	Saturates	ratio
Belut sawah	43.62	39.49	1.10	59.43	40.56	1.47
Jelawat	27.89	36.66	0.76	29.67	41.29	0.72
Kap rumput	28.58	30.22	0.95	51.06	29.57	1.73
Siakap	32.95	45.01	0.73	48.61	34.65	1.40
Tilapia	26.62	44.33	0.60	48.92	39.08	1.25

*Polyunsaturates stated above did not include C18:1

$$\# \text{ Ratio} = \frac{\Sigma\% \text{ Polyunsaturates}}{\Sigma\% \text{ Saturates}}$$

observation is in accordance with the proposed high PUFA content in the phospholipid fraction of fish tissues (Henderson and Toche 1987) and marine animals (Goodnight *et al.* 1982).

CONCLUSION

This study suggests that the high fat content found in Malaysian freshwater fish makes it a good dietary item because of the significant amount of polyunsaturated fatty acids. Another interesting finding of this study was the high amount of DHA found in belut sawah. The results of this study should encourage the Malaysian public to eat more freshwater fish, particularly belut sawah, heeding the advice that "comsumption of as little as one or two fish dishes per week may be of preventive value in relation to coronary heart disease" (Kromhout *et al.* 1985).

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COMMUNICATION I

Pectinesterase Extraction from Guava

ABSTRAK

Pektinesterase telah diekstrak daripada buah jambu (Vietnamese var.) dan diasai. Didapati kepekatan NaCl dan pH mempengaruhi proses ekstraksi pektinesterase daripada buah ini. Nilai ekstraksi paling tinggi iaitu 2.5 mikro mol COOH/min/ml ekstraksi kasar diperolehi menggunakan larutan NaCl 1.75M pada pH 8.0.

ABSTRACT

Pectinesterase (PE) was extracted from guava (Vietnamese variety) fruit and assayed. pH and NaCl concentration influenced the extraction process of PE from this fruit. The highest PE extraction value at pH 8.0 and with 1.75M NaCl solution was 2.5 micro-equivalent COOH/min/ml crude extract.

INTRODUCTION

The suspended material of fruit and vegetable juices, commonly referred to as "cloud", is an unstable colloidal system and its breakdown affects the appearance and quality of the product. The enzyme pectinesterase (PE) is considered to be the causative agent of cloud loss. Due to the action of PE, pectin is de-esterified and then coagulated by Ca^{++} ions in the juice. As a result, loss of cloud occurs and the juice separates into a clear supernatant and a layer of sediment. Therefore it is of interest to purify the PE from guava fruit to see its effect on cloud loss. But an effective method of extracting this enzyme from guava is necessary before its purification. In this work a study is described to investigate the effect of pH and NaCl concentration (extractant) on the extractability of pectinesterase from guava (*Psidium guajava* L.) fruit.

MATERIALS AND METHODS

Extraction of Enzyme.

Fruits were washed, dried and cut into small pieces. The ratio of the fruit to extractant was 1:2. Fruits were blended with either deionised water or NaCl solution of various concentrations (0.25M - 2.0M) in a Waring blender. Crude PE was extracted by incubating the homogenate at pH 7.0, 7.5 and 8.0 in a cold room (at 4°C) for two hours. The pH was adjusted by addition of 2M NaOH or 2M HCl solution. The homogenate was squeezed through muslin cloth and the extract was centrifuged at 15,000 x *g* for 10 minutes at 4°C. The supernatant was used as a crude PE extract.

Pectinesterase Activity

The PE activity was determined by the method described by Kertesz (1955). Briefly, the method consists of titrimetric measurement of the rate of release of carboxyl groups from 1% pectin in 0.1M NaCl solution at pH 7.5 and 30°C. One unit is defined as the activity corresponding to the release of 1 micro-mole carboxyl groups per minute.

RESULTS AND DISCUSSION

The results in *Fig. 1* are the average of duplicates showing the effect of pH and NaCl concentration on the extraction of PE from guava fruit. It was found that both pH and NaCl concentration influenced PE extraction. The results obtained in this study show that as the pH of the extraction medium was increased from 7.0 to 8.0, the optimum NaCl concentration for PE extraction shifted from 1.0M to 1.75M. When PE was extracted at pH 8.0 the optimum NaCl concentration was found to be 1.75M. Increasing the salt concentration up to 2.0M or decreasing it to zero concentration (extraction in pure water), lowered the extraction values. Similar results were obtained for pH 7.0 and 7.5. As a result pH 8.0 was found to be the best for PE extraction from guava at a NaCl concentration of 1.75M (2.5 units) followed by 1.5M (2.1 units). However, pH above 8.0 has not been recommended for PE extraction because excessively high pH inactivates the enzyme (Kertesz 1955).

These results differ from those obtained by Al-Delaimy and Ali (1969) who found that apple

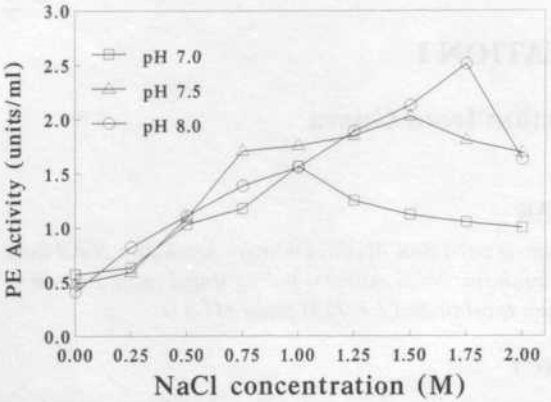

Fig. 1: Effect of pH level and NaCl concentration on PE extraction from guava fruit.

PE was best extracted at pH 7.5 and with 1.5M NaCl solution. Pressay and Avants (1972) and Rillo *et al.* (1992) extracted PE from tomato and mandarin orange by using 1.0M NaCl adjusted to pH 6.0 and 8.0, respectively. On the other hand, orange PE was successfully extracted with 0.25M NaCl solution at pH 7.0 (Korner *et al.* 1980). The relationship of pH and NaCl concentration on PE extraction is not clearly understood. In the extraction of PE from different fruits and vegetables such as apple (Al-Delaimy and Ali 1969), tomato (Pressay and Avants 1972), orange (Korner *et al.* 1980), mandarin orange (Rillo *et al.* 1992) and papaya (Fayyaz *et al.* 1993), different responses to the pH and NaCl concentration of the extraction medium were shown. From these results it may be concluded that this variation may be due to the differences in the texture and maturity of the fruit and it seems advisable to develop and optimise an extraction procedure for a given fruit.

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A. FAYYAZ and B. A. ASBI

Department of Food Technology,
Faculty of Food Science and Biotechnology,
Universiti Pertanian Malaysia,
43400 Serdang, Selangor, Malaysia.

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COMMUNICATION II

Insect Pests of Grapes in Malaysia

ABSTRAK

Serangga yang memakan pelbagai bahagian pokok anggur di Semenanjung Malaysia telah dipungut dan dikaji. Enam spesies serangga perosak dan satu spesies hamama telah dicatatkan: terdiri dari 4 spesies yang memakan pucuk dan daun, 2 spesies pengorek batang dan dahan, dan 2 spesies yang menyerang buah. Spesies perosak terdiri daripada Apogonia cribricolla *Burmeister,* Hypomeces squamosus *Fabricius,* Nipaecoccus viridis *(Newstead).* Protaetia acuminata *Fabricius,* Vespa tropica *Linnaeus,* Xylosandrus compactus *(Eichoff). Kumbang Scolytid* Xylosandrus compactus *merupakan serangga perosak yang paling merbahaya kerana boleh mengakibatkan dahan utama anggur mati. Spesies hamama yang menyerang anggur ialah* Eutetranychus *sp. Secara amnya, penanaman anggur di Malaysia tidak menghadapi masalah serangan perosak yang serius.*

ABSTRACT

All insects attacking various parts of grape plants were collected and studied. Six insect pests from 5 families and one mite species were recorded; four species were leaf feeders, two species were stem and vine borers and two species infested fruits. The insect pests were Apogonia cribricolla *Burmeister,* Hypomeces squamosus *Fabricius,* Nipaecoccus viridis *(Newstead),* Protaetia acuminata *Fabricius,* Vespa tropica *Linneaus, and* Xylosandrus compactus *(Eichhoff). Scolytid borer* Xylosandrus compactus *was the most serious pest as it killed part of the main vines. The mite species was Tetranychidae,* Eutetranychus *sp. However, grape planting did not face serious insect problems.*

INTRODUCTION

Grape cultivation in Malaysia is still at the research stage. It began at Universiti Pertanian Malaysia in 1981 as a study on the feasibility of growing table grapes commercially. Earlier tests had been made on a variety for making preserves but not for table consumption (Chan *et al.* 1975). From 1981-87 experiments at UPM concentrated on table varieties such as White Malaga. Later other varieties were brought in from the U.S.A., including varieties such as Cardinal, Early Muscat, Emperor, Isabella. In Peninsular Malaysia, it is a demanding crop agronomically, and also requires intensive spraying throughout the year especially for protection against diseases. A similar situation is experienced in Indonesia (Rismunandar 1984; Setiadi 1988).

Information regarding commercial grape plantings in Malaysia is still scanty although some plantings have been made by farmers. This paper is intended to provide information on the pests associated with grapes in Peninsular Malaysia.

MATERIALS AND METHODS

Insect Sampling

Insects were collected either manually from the plants, mulches, and the soil near the vines or by rearing from infested vines. All field-collected insect pests were then preserved and some species were sent to the International Institute of Entomology, London for species confirmation.

RESULTS AND DISCUSSION

Grape plants were found to be susceptible to diseases caused by insects found on the grapes. A total of six families of arthropod pests comprising six species of insects and a mite species were recorded (Table 1). Among the species, four were foliage feeders, two were stem and vine borers and two were fruit feeders.

Leaf Pests

Beetles are among the common leaf pests normally found underneath the mulches or pebbles beneath the vines. They are general leaf feeders, and on grapes the damage is seen on old and young leaves. Damage can result in slow growth of the plants. Three species commonly found on grapes, *Protaetia acuminata, Apogonia cribricolla* and *Hypomeces squamosus,* can cause defoliation of the plant.

The red spider mite, *Eutetranychus* sp., is commonly found on the lower side of the leaf, feeding usually on the interveinal areas. Spider mite problems are sporadic, occurring generally in the dry spells and the population can be high

TABLE 1
Arthropod pests of grapes in Peninsular Malaysia.

Pest species	Leaf	Stem/vines	Fruits
COLEOPTERA			
Scarabaeidae			
Apogonia cribricolla Burm.	+		
Protaetia acuminata Fabr.	+		
Cucurlionidae			
Hypomeces squamosus Fabr.	+		
Scolytidae			
Xylosandrus compactus (Eich.)		+	
HOMOPTERA			
Pseudococcidae			
Nipaecoccus viridis (News)		+	+
HYMENOPTERA			
Vespidae			
Vespa tropica Linn.			+
ACARINA			
Tetranychidae			
Eutetranychus sp.	+		

when water stress occurs. They are very common on varieties such as Cardinal and White Malaga.

Stem and Vine Pests

The beetle, *Xylosandrus compactus* (adult and larva), is injurious to the trunks and vines. The damage is visible as punctured holes with frass deposits at the opening. The larvae bore longitudinal galleries in the pith; damaged vines will eventually die. The larvae pupate inside the galleries. Emerging beetles tunnel into the pith and cause more damage to the older established vines.

Fruit Pest

Another serious problem faced by grape growers is the attack by the wasp, *Vespa tropica*, when the fruits begin to ripen. Ripening fruits are attractive to wasps and birds. Damage is caused when the wasps bite the fruits on the bunch causing them to rot. Damage can be extensive enough to cause alarm to the grower.

The family Pseudococcidae includes several species of mealybugs which are known pests of many fruits including grapes. The species associated with grapes in Malaysia is *Nipaecoccus viridis* which is found on aerial roots and fruit bunches. This polyphagous species of mealybug is common throughout Southern Asia and attacks a variety of fruit crops. The practice of bagging to protect fruits against birds and wasps actually encourages mealybug infestation. Once within the bag the mealybug is sheltered from rain and natural enemies, and secretes a sticky exudate which downgrades the grapes. The infestation of mealybug is not normally very serious.

Other pests of fruits include various species of birds. The birds seem to damage fruit bunches which are about to mature and ripen. The damaged fruits left on the bunch are infected by bacteria. The sap from the damaged fruits spreads to the neighbouring fruits on the bunch which leads to infection and rotting of fruits; eventually the whole bunch is damaged.

CONCLUSION

Grape plants are attacked by many arthropod pests, which can be grouped into root, leaf and fruit pests (Bournier 1976). *Xylosandrus compactus* can cause serious damage to the main vines. Only perfect grapes are competitive and saleworthy; so it is particularly important to protect them from pests, diseases and weeds (Bayer 1989).

ROHANI IBRAHIM

Department of Plant Protection
Faculty of Agriculture
Universiti Pertanian Malaysia
43400 UPM, Serdang
Selangor Darul Ehsan, Malaysia

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