Effect of Maturity on Chlorophyll, Tannin, Color, and Polyphenol Oxidase (PPO) Activity of Sugarcane Juice (Saccharum officinarum Var. Yellow Cane)

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · MARCH 2002

Impact Factor: 2.91 · DOI: 10.1021/jf010959l · Source: PubMed

4 AUTHORS, INCLUDING:

Russly Abdul Rahman
Putra University, Malaysia
182 PUBLICATIONS **1,578** CITATIONS

SEE PROFILE

Effect of Maturity on Chlorophyll, Tannin, Color, and Polyphenol Oxidase (PPO) Activity of Sugarcane Juice (*Saccharum officinarum* Var. Yellow Cane)

HANAN YASSIN M. QUDSIEH,[†] SALMAH YUSOF,*[,†] AZIZAH OSMAN,[‡] AND RUSSLY ABDUL RAHMAN[†]

Department of Food Technology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

A study was conducted to determine the effect of sugarcane maturation on the contents of chlorophyll, tannin, and polyphenol oxidase (PPO) activity and on color change of sugarcane juice. The maturation period of the cane studied was between 3 and 10 months after planting. Different parts of the cane, namely, the top, middle, and bottom portions, were analyzed. Results obtained indicated that there were significant ($P < 0.01$) decreases in total chlorophyll *a* and *b* and tannin contents during maturity followed by slower rates of decrease of both parameters at the end of maturity stages. There were no significant differences ($P > 0.05$) in chlorophyll and tannin contents between the middle and bottom portions. On the other hand, the top portion of the stem had a significantly ($P < 0.01$) lower concentration of chlorophyll and a significantly ($P < 0.01$) higher content of tannin. PPO activity of sugarcane juice was determined using chlorogenic acid as a substrate. There was a highly significant difference ($P < 0.01$) in PPO activity of cane juice during maturity. PPO activity was high at the early development stage, decreased during maturation, and then remained relatively constant at the end of maturity. PPO activity was higher when chlorogenic acid was used as substrate. There were also significant differences ($P < 0.01$) in juice color (L^*, a^*, b^* values) from different portions at different maturity stages. At the early stages, the color of extracted juice was dark, and then the juice turned to yellowish green during maturity. The decrease in green color or the increase in the yellow color could be associated with the decline in chlorophyll. The overall color change (ΔE) at maturity indicated that the color of the middle and bottom portions was lower than that of the top portion.

KEYWORDS: Sugarcane juice; maturity; polyphenols; chlorophyll; PPO

INTRODUCTION

In many parts of the world sugarcane juice is mainly used for sugar production. However, in Malaysia freshly extracted sugarcane juice is a very popular beverage among the population for its sweet taste, favorable flavor, and color. The juice is extracted from a cane variety grown especially for juice production known as "Tebu Kuning" (or Yellow Cane) (*1*). Sugarcane juice is currently sold in retail amounts by small operators at public eateries or in supermarkets.

Sugarcane juice contains a series of coloring matters such as chlorophyll and polyphenolic compounds (*2*), and these determine the color and in turn the acceptability of sugarcane juice. Previous work (*3*, *4*) has shown that sugarcane juice tended to change color immediately after extraction. It has been suggested that the color development was due to an enzymatic browning reaction brought about by the enzyme polyphenol oxidase (PPO) acting upon the phenolic compounds (*5*). According to an earlier publication (*6*), the color of sugarcane juice is related to the concentration of phenolic compounds; it becomes darker when they react with the PPO enzyme. Chlorophyll might also contribute to the attractive fresh greenish appearance of sugarcane juice. In an earlier work (*4*) it was found that the change in the color of sugarcane juice was partly due to the decrease in chlorophyll content.

To date there is practically no published information regarding the changes in chlorophyll, tannin, and PPO activity and color development of sugarcane juice of the yellow cane variety during development and maturation. Therefore, this study was conducted to determine the effects of maturation on the above components. Results of this study may be useful to the growers, juice processors, and vendors in their effort to curb the objectionable color development process.

MATERIALS AND METHODS

Sample Source and Sampling. Sugarcanes were obtained from a farm in Semenyih, Selangor. Eight hundred sugarcane plants were tagged randomly 1 month after planting. Three stems were harvested

* Author to whom correspondence should be addressed (fax +603-89423552; e-mail salmahy@putra.upm.edu.my).
[†] Department of Food Technology.
[‡] Department of Food Science.

monthly starting from 2 months after tagging. The harvested stems were immediately transported to the laboratory at the Faculty of Food Science and Biotechnology, Universiti Putra Malaysia. Then each stem was divided into top, middle, and bottom according to the node number. The experiment was performed in triplicate.

Extraction of Juice. Before extraction of juice, the canes were cleaned and washed to remove dirt and foreign particles from the surfaces. A three-roller power crusher was used to extract the juice. The juice was then filtered using a four-layer muslin cloth and chilled immediately at 4 °C to slow physical and chemical changes.

Determination of Chlorophyll. Chlorophyll was determined by using a spectrophotometric method (*7*), and the amount of chlorophyll was calculated on the basis of spectrophotometric equations (*8*). Fifty milliliters of freshly extracted sugarcane juice was filtered under half atmospheric pressure vacuum on a Büchner funnel through fiberglass (0.45 μm). The residue was then placed in a 15 mL centrifuge tube, and 15 mL of prechilled (4 °C) acetone (99%) was added. The mixture was shaken thoroughly and allowed to stand overnight in a dark place. The next day, the sample was centrifuged using a refrigerated centrifuge (Beckman, model J2-21 M/E) at 4000 rpm for 10 min. The supernatant was decanted into a spectrophotometer quartz cuvette, and the absorbance was measured at 664, 647, and 630 nm. The formulas used to calculate the amount of chlorophyll were

$$\text{chlorophyll } a = 11.85\ \text{OD } 664 - 1.54\ \text{OD } 647 - 0.08\ \text{OD } 630$$

$$\text{chlorophyll } b = 21.03\ \text{OD } 647 - 5.43\ \text{OD } 664 - 2.66\ \text{OD } 630$$

$$\mu\text{g of chlorophyll/L} = Cv/10V$$

where v is the volume of acetone in mL (15 mL), V is the volume of sugarcane juice in L, chlorophylls a and b are the chlorophylls substituted for C in the above equation, and C is the value of chlorophyll a plus the values of chlorophyll b.

Determination of Tannin Content. One milliliter of sugarcane juice was diluted with 100 mL of distilled water and then filtered. Folin−Denis reagent was used as reagent, and total polyphenolic content was determined according to the AOAC method (*9*). Tannic acid was used as the standard, and solutions containing 0−10 mL aliquots were prepared to plot the calibration curve. The samples were measured for blue intensity using a U-2000 Hitachi spectrophotometer at 760 nm.

Determination of PPO Activity. The enzyme extraction method used was based on the same method reported earlier (*10*). One hundred milliliters of prechilled sugarcane juice was added to 100 mL of 0.02 M phosphate buffer (pH 6.8) containing 1.5% polyvinylpyrrolidone (PVP) and 0.5% Triton X-100. The suspension was filtered through four layers of cheesecloth and the filtrate centrifuged at 12000 rpm for 15 min at 4 °C in a refrigerated centrifuge (Beckman, model J2-21). Enzyme activity was determined by measuring the increase in the absorbance at 410 nm using a spectrophotometer (U-2000 Hitachi). The reaction mixture contained 3.5 mL of 0.20 M phosphate buffer (pH 6.8), 1 mL of 0.05 M catechol, and 0.5 mL of enzyme solution in a final volume of 5 mL. The rate of the reaction was calculated from the initial linear slope of activity curves. The enzyme unit was defined as the change in absorbance of 0.001 per minute under the condition of the assay (*10*). PPO activity was measured immediately after the extract was obtained.

Determination of Juice Color. The color of sugarcane juice was determined by using a Hunter Lab Colorimeter Ultra-Scan, model SN 7877. The values were extracted as lightness L^*, redness a^*, and yellowness b^* readings (*11*). The overall color change during maturity was calculated using the equation $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$ (*12*).

Statistical Analysis. Data were analyzed by the analysis of variance and Duncan's multiple-range test using a Statistical Analysis System (SAS) (*13*) program.

RESULTS AND DISCUSSION

Chlorophyll. There was a major change in chlorophyll during the early stage of cane development. As shown in **Figure 1**, chlorophyll content decreased significantly from 160 to 48.64 μg/L from the third to the fourth month. This was followed by a slower decrease up to the seventh month (41.53 μg/L). From then on the values continued to decline further until the end of the maturity stages. The juice obtained from the top portion of the stem had a significantly ($P < 0.05$) lower concentration of chlorophyll (45.14 μg/L) than the middle (52.11 μg/L) and bottom portions (53.12 μg/L). No significant difference ($P > 0.05$) was found between the middle and bottom portions.

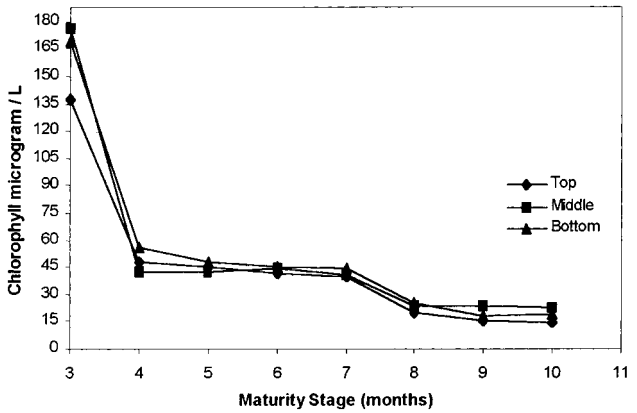

Figure 1. Changes in chlorophyll content of sugarcane juice harvested at different stages of maturity.

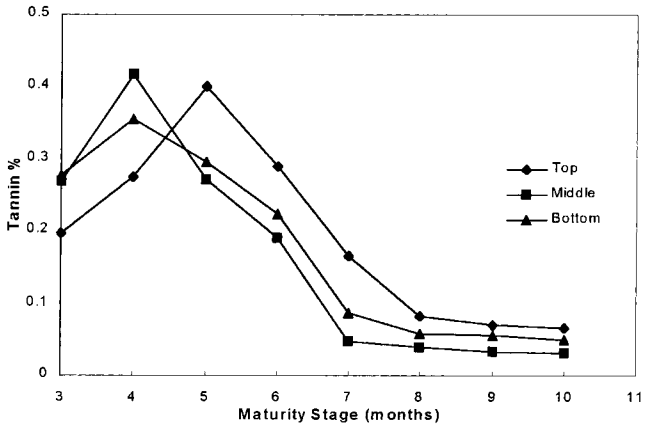

Figure 2. Changes in tannin content of sugarcane juice harvested at different stages of maturity.

Tannins. Polyphenols are often found in plants and are common constituents of many fruits, vegetables, and some beverages (*14*). Tannin was one of the polyphenol groups believed to contribute to the color and astringent sensation in fruits and fruits juices (*11*, *15*).

The results obtained indicated that there was highly significant difference ($P < 0.01$) in the tannin content of yellow cane juice from different portions when harvested at the different maturity stages (**Figure 2**). Tannin content decreased rapidly up to the seventh month and then decreased slowly at the end of the maturity stage. At 3 months old, tannin content was 0.24%, whereas at the end of the maturity stage, tannin content decreased to 0.025%. It was observed that a significant ($P < 0.01$) decrease in tannin content started 4 months after planting (0.32%) until 7 months (0.098%). This was followed by a continued decline at the end of the maturity stages. This result revealed that tannin content was present in relatively high quantities in the immature canes, and even when the canes attained maturity, the amount of polyphenolic substances present remained high.

PPO Activity. The results obtained in this work indicated that there was a highly significant difference ($P < 0.01$) in PPO

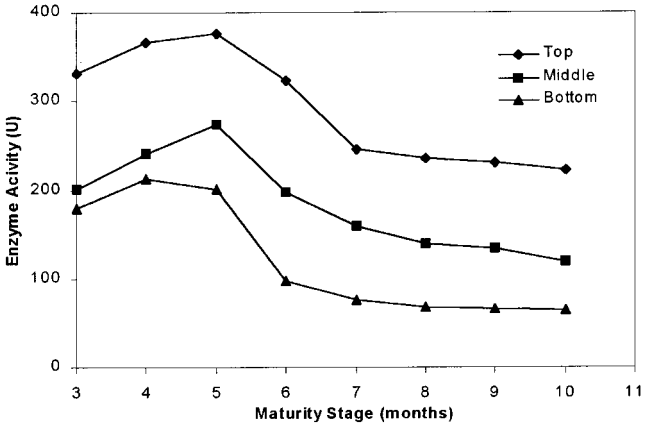

Figure 3. Changes in PPO activity of sugarcane juice harvested at different stages of maturity.

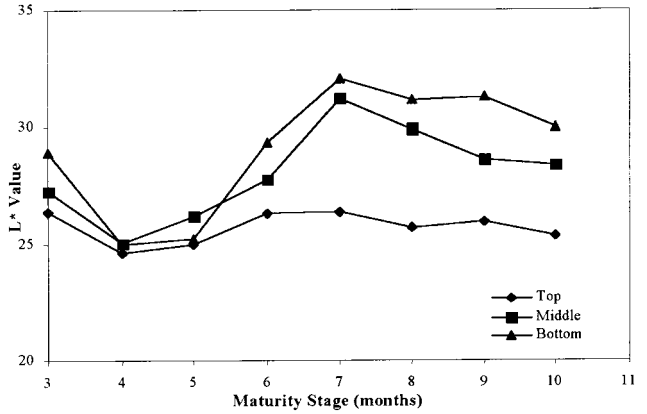

Figure 4. Changes in Hunter *L** values of sugarcane juice harvested at different stages of maturity.

activity of yellow cane juice taken from different portions when harvested at different maturity stages. The top portion of the stem had the highest ($P < 0.01$) activity compared to the middle and bottom portions. As can be seen in **Figure 3**, there was an initial increase in PPO during the first 3 months (3−5 months) of sugarcane development. PPO was high at the early development stage, decreased during maturation, and then remained relatively constant at the end of the maturity stages. There was a high negative correlation ($r = -0.85$; $P < 0.0001$) between PPO activity and cane maturity. Results from the present study further indicated that PPO activity was higher when chlorogenic acid was used as substrate compared with catechol.

Color of Juice. A significant difference ($P < 0.01$) was observed in the color of juice at different stages of maturity. As can be seen in **Figure 4**, the *L** (lightness) values were low during the third, fourth, and fifth months, indicating that the juice had a dark color at the early stages of maturity. The average values were 27.56, 24.91, and 25.50, respectively (**Table 1**). The *L** value increased during the maturity stages until it reached a maximum value of 29.93 at the seventh month, and after that it decreased slowly until the end of maturation (*L** value = 28.71 at 10 months). The change in *L** values might be due to the decrease in the intensity of green color (due to chlorophyll) as the plants became mature. There were also significant differences ($P < 0.01$) in the juice color within the stem portions (top, middle, and bottom). The juice from the bottom portion gave the highest average *L** value (29.12) followed by the middle portion (28.09) and the top portion (25.73) (**Table 1**).

Table 1. Mean Values and Standard Deviation for Juice Color (Hunter *L**, *a**, *b** Values) of Yellow Sugarcane during Development and Maturation[a]

	Hunter values		
main effect	*L**	*a**	*b**
maturity stage (months)			
3	27.56 ± 1.57^{c}	3.10 ± 0.50^{e}	5.27 ± 0.63^{f}
4	24.91 ± 0.81^{d}	4.65 ± 0.81^{a}	7.01 ± 0.13^{a}
5	25.50 ± 0.85^{c}	4.60 ± 0.65^{a}	6.71 ± 0.60^{abc}
6	27.84 ± 1.70^{c}	4.35 ± 0.60^{b}	6.73 ± 1.5^{ab}
7	29.93 ± 2.14^{a}	4.00 ± 0.64^{c}	6.62 ± 1.88^{cb}
8	28.94 ± 2.11^{b}	3.65 ± 0.59^{d}	6.24 ± 1.90^{d}
9	28.59 ± 2.2^{b}	2.79 ± 0.49^{f}	6.36 ± 2.05^{cd}
10	28.71 ± 2.55^{b}	2.49 ± 1.10^{g}	5.75 ± 1.60^{e}
portion of stem			
top	25.73 ± 1.05^{c}	3.96 ± 0.93^{a}	6.79 ± 0.90^{a}
middle	28.09 ± 1.75^{b}	3.77 ± 0.97^{b}	6.20 ± 1.25^{b}
bottom	29.12 ± 2.55^{a}	3.38 ± 1.12^{c}	6.01 ± 1.67^{b}

[a] Means within the same column followed by the same superscript letter are not significantly different at 1% level ($P < 0.01$). Each value represents the average for nine different samples.

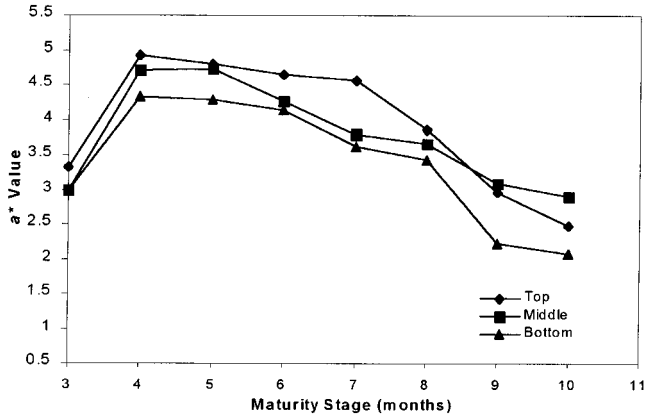

Figure 5. Changes in Hunter *a** values of sugarcane juice harvested at different stages of maturity.

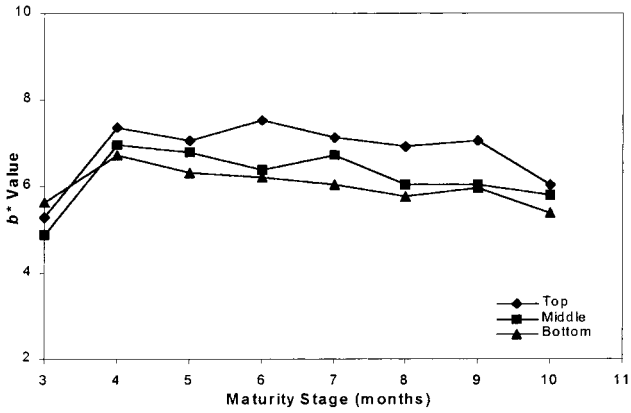

Figure 6. Changes in Hunter *b** values of sugarcane juice harvested at different stages of maturity.

With reference to **Figures 5** and **6**, there were highly significant differences ($P < 0.01$) observed in the *a** value (redness) and *b** value (yellowness) of sugarcane juice from different portions when harvested at different maturity stages. The *a** and *b** values increased significantly at the early stages of maturity, resulting in brown and dark colored juices. The increase in *a** values seemed to correspond with tannin content. When tannin content was high at the early stages of maturity, the juice was brown, as reflected by the high *a** value. The

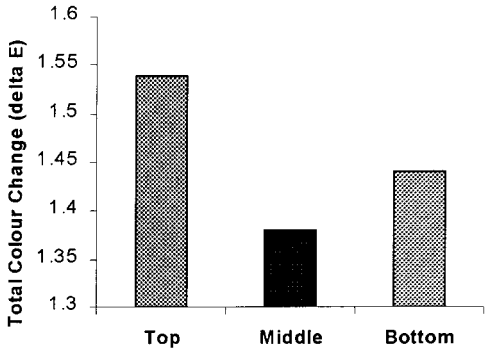

Figure 7. Total color change of sugarcane juice extracted from different portions of cane during the maturity period.

decrease in tannin content was accompanied by a decrease in a^* value, resulting in a decrease in browning, and the juice became less brown and lighter. When PPO was high at 4−5 months, the a^* value was also high. The decrease in PPO activity during maturity was accompanied by a decrease in a^* value, resulting in a decrease in browning. However, PPO has a low positive correlation with high a^* value ($r = 0.55$; $P < 0.02$) but has a high negative correlation ($r = 0.86$; $P < 0.0001$) with L^* value during maturation.

The juice obtained from the top portion was darker and brown compared to that obtained from the middle and bottom portions. From **Figure 3**, the reason for the more intense dark color could be attributed to a higher enzyme activity in the top portion, especially in younger plants. It is also possible that the younger plant contains high amounts of substrate polyphenols as observed in **Figure 2**. Results of this study showed a strong correlation ($r = 0.97$; $P < 0.0001$) between PPO activity and tannin content during maturation. This correlation indicated that the PPO activity was high when the tannin content was also high. It could be that the levels of phenolic compound present determined the extent of PPO activity during maturation. The availability of phenolic compound may determine the extent of the browning reaction in cane juice (*5*).

The juices obtained from the middle and bottom portions had a yellowish green color. From the lightness L^* value, it could be presumed that some biochemical degradation of coloring compounds such as tannin occurred during maturation. This was confirmed by the decrease in tannin content during maturation as shown in **Figure 2**. There were also significant differences ($P < 0.01$) in PPO activity of sugarcane juice within the cane portions (top, middle, and bottom). **Table 1** shows significant differences ($P < 0.01$) in average juice color values within the cane or stem portions (top, middle, and bottom). The overall color change (ΔE) (**Figure 7**) during maturity indicated that the changes in color of the middle and bottom portions were smaller compared to that of the top portion.

LITERATURE CITED

(1) Salunkhe, D. K.; Desai, V. G. *Postharvest Biotechnology of Sugar Crops*; CRC Press: Boca Raton, FL, 1988; pp 35−74.
(2) Bhupinder, K.; Sharma, K. P.; Harinder, K. Studies on the development and storage stability of ready to serve bottled sugarcane juice. *Int. J. Trop. Agric.* **1991**, *9* (2), 128−134.
(3) Bucheli, S. C. Isolation of full-length cDNA encoding polyphenol oxidase from sugar cane, a C4 grass. *Plant Mol. Biol.* **1996**, *31*, 1233−1238.
(4) Yusof, S.; Lee, S. S.; Osman, A. Changes in quality of sugarcane juice upon delayed extraction and storage. *Food Chem.* **2000**, *68*, 395−401.
(5) Bucheli, S. C.; Robinson, P. S. Contribution of enzymic browning to colour in sugarcane juice. *J. Agric. Food Chem.* **1994**, *42*, 257−261.
(6) Patton, N. H.; Duong, M. Sugarcane Phenolics and First Expressed Juice Colour. Part II: Concentration of Phenolics in Sugarcane and Colour of First Expressed Juice. *Int. Sugar J.* **1992**, *94* (1123), 205.
(7) Richard, F. A.; Thomas, G. T. The estimation and characterization of plankton populations by pigment analysis. I. Spectrophotometric method for the estimation of plankton pigments. *J. Mar. Res.* **1952**, *11* (2), 156−159.
(8) Jeffrey, S. W.; Humphrey, G. F. New spectrophotometric equation for determination of chlorophyll *a*, *b* and *c* in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanzen.* **1975**, *167*, 191.
(9) AOAC. *Official Methods of Analysis*, 14th ed.; Association of Analytical Chemists: Washington, DC, 1980.
(10) Augustin, M. A.; Ghazali, H. M.; Hashim, H. Polyphenoloxidase from guava (*Psidium guajava* L.). *J. Sci. Food Agric.* **1985**, *36*, 1259−1265.
(11) Ranganna, S. *Manual of Analysis of Fruits and Vegetables Products*; McGraw-Hill: New Delhi, India, 1977; pp 1−20.
(12) Sankat, K. C.; Basanta, A.; Maharaj, V. Light mediated red colour degradation of the pomerac (*Syzygium malaccens*) in refrigerated storage. *Postharvest Biol. Technol.* **2000**, *18*, 253−257.
(13) SAS. *SAS User's Guide: Statistics*, version 5 ed.; SAS Institute: Cary, NC, 1985.
(14) Gaulejac, S. N.; Vivas, N.; Freitas, V.; Boureois, G. The influence of various phenolic compounds on scavenging activity assessed by an enzymatic method. *J. Sci. Food Agric.* **1999**, *79*, 1081−1090.
(15) Manchado, S. P.; Cheynier, V.; Moutounet, M. Interaction of grape seed tannin with salivary proteins. *J. Agric. Food Chem.* **1999**, *47*, 42−47.

Received for review July 23, 2001. Revised manuscript received December 3, 2001. Accepted December 5, 2001. We are grateful to the Ministry of Science, Technology and Environment, Malaysia, for providing the fund to Universiti Putra Malaysia to carry out this work.

JF010959L